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(54) Title: MORPHATIDES: NOVEL SHAPE AND STRUCTURE LIBRARIES

(57) Abstract

This invention provides a method for identifying complexes from a library, wherein said complexes are selected for their ability to perform a preselected or desired function on a target molecule or by having a preselected structure, each complex being designated a morphatide, said method comprising: (a) preparing a library of morphatides, comprised of: (i) a scaffolding nucleic acid having one or more regions or randomized sequence; (ii) linker components; and (iii) agent molecules; (b) screening the library of morphatides prepared in step (a) by contacting, binding, or associating the morphatides with suitable target molecules; (c) separating the morphatides from the library of morphatides and target molecules; thereby identifying complexes selected for their ability to perform a preselected or desired function on a target molecule or by having a preselected structure.

MORPHATIDES: NOVEL SHAPE AND STRUCTURE LIBRARIES

Throughout this application, various references are referred to
within parentheses. Disclosures of these publications in their
10   entireties are hereby incorporated by reference into this
application to more fully describe the state of the art to which
this invention pertains. Full bibliographic citation for these
references may be found at the end of this application, preceding
the claims.
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BACKGROUND OF THE INVENTION

The present invention relates to the production and screening of
libraries of compounds and, more particularly, to the generation
20   and screening of shape and structure libraries produced from
large or small size molecules for the purpose of identifying
potentially useful agents.

New agents for effectively modulating a range of biological
25   processes have a variety of applications in industry, medicine
and agriculture. The identification of structurally unique lead
compounds is an important step in selecting such biologically
useful agents. Historically and currently, mass screening of
collections of large numbers of molecules (chemicals or other
30   compounds) and mixtures of molecules, has been the most
successful approach for identifying lead compounds. Most of
these collections are either compound databases generated by
pharmaceutical research, natural products collections, such as
fermentation broths, or more recently, collections of peptides,
35   nucleotides or other synthesized molecules.

Each of these collections, or libraries, has its advantages as
well as its limitations. Collections generated via research,

such as compound databases, can obtain a potentially limitless
repertoire of compounds for search (large numbers), however they
tend to contain a limited number of diverse structures,
representing only a small portion of the total structural
5   diversity possibilities. Natural product libraries can offer
structural complexity, however the difficulty in downstream
manufacturing of these products, and of reducing leads to useful
products is a serious limitation of this type of approach.
Peptide libraries are limited to peptides or peptide mimics.
10   There has been limited success in the conversion of peptide
chemical leads into pharmaceutically useful drug candidates.
These lead compounds are at a disadvantage for generating orally
active drug candidates due to the complexity of determining their
three dimensional structures for synthesis of small organic
15   molecules, and due to the sensitivity of their peptide bonds to
acid hydrolysis. However, the structural diversity offered by
this technology is its greatest advantage. Nucleotide libraries
are also restricted to the genetic repertoire (nucleotides) or
nucleotide analogues that preserve specific Watson-Crick pairing
20   and can be copied by a polymerase, hence they are more limited
in their useful structural diversity than peptide libraries,
however this remains an advantage of these libraries. Nucleotide
libraries also offer the capacity for cloning and amplification
of DNA sequences, which allows for enrichment by serial selection
25   and provides a facile method for decoding the structure of active
molecules.

Compound databases have historically been generated via the
chemical modification of existing compounds to generate analogs,
30   which then follow the conventional paradigm of small molecule
lead development in which a compound undergoes many rounds of
individualized, hand-crafted modification and biological testing
en route to drug candidacy. Natural product libraries are derived
from collections of natural materials, such as fermentation
35   broths, plant extracts, etc.

Peptide and nucleotide libraries are generated by sequence

2

randomization of individual monomers using a single naturally existing biological linkage (3'-5' phosphate linkage of nucleotides or amide linkage of peptides). As indicated, the biggest advantage in using peptide and nucleotide libraries is

5   the apparent structural diversity afforded with the technologies. For example, Figure 1 briefly demonstrates one well known strategy for generating and utilizing Aptamers, a library of nucleotide shapes.

10   For the discovery of drugs and other commercially valuable compounds, small molecule, highly complex libraries containing diverse functionalities have the greatest utility and provide the greatest chance of success. Libraries must also permit identification and evaluation of the structure/activity

15   relationship of the potentially small fraction of active molecules among the larger number of inactive or less active compounds. To satisfy these needs, recent trends are to generate chemical libraries and new techniques to evaluate and screen them. Chemical libraries have been defined as intentionally

20   created collections of differing molecules which can be prepared synthetically or biosynthetically. A type of synthetic strategy which can lead to large chemical libraries is combinatorial chemistry. Combinatorial chemistry has been defined as the systematic and repetitive, covalent connection of a set of

25   different `building blocks' of varying structures to each other to yield a large array of diverse molecular entities. (Gallop, M.A. et al., 1994) Building blocks can include nucleotides, carbohydrates, peptides or peptoids into ordered structures. Chemical libraries generated utilizing combinatorial chemistry

30   can display remarkable diversity. These large libraries can be selected for potential pharmacological activity by their affinity to specified ligands. Several groups have taken advantage of these facts to develop systems utilizing modified and unmodified oligonucleotides and modified and unmodified polypeptides as

35   ligands to bind targets. Many examples are available in the art, a few of which are described herein.

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Although oligonucleotides inherently have fewer potential building blocks to provide diversity, they have demonstrated a remarkable affinity for selected targets. Examples include both
5   single-stranded RNA and single- and double-stranded DNA. A great attraction of nucleic acid based combinatorial chemistry is the potential for directed evolution. Repeated cycles of selection for the highest affinity and error-prone PCR can lead to increased diversity and oligomers with an even greater affinity.
10
The versatility of the binding capabilities of DNA and RNA oligonucleotides seems inexhaustible and the growing number of applications are a tribute to its enormous potential. One of the earliest polynucleotides of this type was directed to the human
15 blood clotting enzyme thrombin (Bock et al., 1992). This study initiated a search for other thrombin inhibitors based on this approach (Bracht and Schroer, 1994; Kubik et al., 1994; Griffin et al., 1993). Numerous other polynucleotide sequences have been selected from initially random libraries of molecules. Examples
20 include DNA and RNA oligomers selected against HIV integrase (Allen et al., 1995), its Rev protein (Giver et al., 1993; Tuerk and MacDougal-Waugh, 1993; Jensen et al., 1994; Jensen et al., 1995; Bartel et al., 1991), and its reverse transcriptase (Chen and Gold, 1994; Tuerk et al., 1992; Schneider et al., 1995), as
25 well as against reverse transcriptase of feline immunodeficiency virus (Chen et al., 1996). Other were developed against human growth factors, such as nerve GF (Binkley et al., 1995), vascular endothelial GF (Jellinek et al., 1994), and basic fibroblast GF (Jellinek et al., 1996; Jellinek et al., 1995) and against Qβ
30 replicase (Brown and Gold, 1995a; Brown and Gold, 1995b). An RNA oligomer has been made against nucleolin (Ghisolfi-Nieto et al., 1996), an essential protein in ribosome biosynthesis. Oligomers against selectins may show potential in treatment of anti-inflammatory diseases (O'Connell et al., 1996). Other proteins

4

against which these oligomers have been selected include immunoglobulin IgE (Wiegand et al., 1996), bacteriophage T4 DNA polymerase (Tuerk and Gold, 1990), bacteriophage R17 coat protein (Schneider et al., 1992), the E. coli rho factor (Schneider et
5   al., 1993), leucine receptive regulatory protein (Cui et al., 1995) and several ribosomal proteins (Dobbelstein and Shenk, 1995; Ringquist et al., 1995).

These polynucleotides can also be selected for their affinity to
10 small molecules. These include early experiments which demonstrated RNA oligomers that bind specifically to a variety of dye molecules (Ellington and Szostak, 1990). Later this finding was extended to DNA oligomers (Ellington and Szostak, 1992). Interestingly, the sequences of these DNA and RNA species
15 are quite distinct, even when selected for the identical substrates.

The oligopeptide substance P, a mammalian neuro-transmitter, was used to select RNA molecules with high affinity against the
20 neurotransmitter by Nieuwlandt et al. (1995). Single amino acids and other small molecules are also able to bind such molecules. Examples include valine (Majerfeld and Yarus, 1994), arginine (Yarus and Majerfeld, 1992; Puglisis et al., 1992; Nolte et al., 1996; Hicke et al., 1989; Geiger et al., 1996; Burgstaller et
25 al., 1995), citrulline (Burgstaller et al., 1995), ATP (Huizinga and Szostak, 1995; Sassanfar and Szostak, 1993), adenosine (Huizinga and Szostak, 1995), D-adenosine (Kluszmann et al., 1996), flavin mono-nucleotide (Fan et al., 1996), theophylline (Jenison et al., 1994), cyanocobalamine (Lorsch and Szostak,
30 1994).

Another interesting potential of these oligomers was pursued by Morris et al. (1994) who tried unsuccessfully to select for a molecule specific for a reaction transition state, effectively

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attempting to create a catalyst. Hale and Schimmel (1996), however, did succeed in generating a DNA molecule that induces hydrolysis of a misactivated amino acid bound to a tRNA synthetase, a case of protein synthesis editing. Lorsch and
5   Szostak (1994) succeeded in selecting for several RNA aptamers with 2' or 5' polynucleotide kinase activity.

Polynucleotides with modifications of incorporated nucleotides have been selected by Latham et al. (1994), who incorporated 5-
10 (1-pentynyl)-2'-deoxyuridine into thrombin binding DNA molecules. The primary sequence of these modified DNA oligomers was strikingly different from the unmodified DNA molecule.

The use of nucleic acids for therapeutic and diagnostic
15 applications often requires their stability in biological fluids. Aside from chemical modification, nuclease-resistant ligands can be generated by using L-ribose-based nucleotides (Nolte et al. 1996, Klussmann et al. 1996). In this approach the conventional D-RNA directed against the optical mirror image of the target is
20 selected first using repeated rounds of mutation and selection of the nucleic acid and subsequently the corresponding L-RNA is chemically synthesized. L-RNA's with specificity for L-arginine (38-mer, Kd=60 mM, Nolte et al 1996) and D-adensosine (58-mer, Kd=1.7 mM, Klussmann et al. 1996) have been isolated and shown
25 to be stable in human serum at 37°C. Another example includes chirally pure methylphosphonate linkages that are suitable for generating oligomers capable of efficiently hybridizing with DNA or RNA and are highly resistant to metabolic breakdown in biological systems (Reynolds et al. 1996).
30
Another interesting method for the selection of nucleic acid molecules with highly specific binding to target molecules has been developed and termed "SELEX" (Systematic Evolution of Ligands by EXponential enrichment), which is described in U.S.

Patent No. 5,270,163 entitled "Nucleic Acid Ligands" and in PCT/US91/04078. SELEX is a method for making a nucleic acid to a desired target molecule involving the selection from a mixture of candidate oligonucleotides and the step-wise iteration of
5   binding, partitioning and amplifying, using the same general selection scheme, to achieve a desired criterion of binding affinity and selectivity. The basic SELEX method has also been modified to achieve a number of specific objectives. (For instance, those described in PCT/US94/10562 filed September 19,
10   1994, and WO 96/09316 filed September 19, 1995).

For example, SELEX has been used in conjunction with gel electrophoresis to select nucleic acid molecules with specific structural characteristics, such as bent DNA; as a method for
15   selecting nucleic acid ligands containing photoreactive groups capable of binding and/or photo-crosslinking to and/or photoinactivating a target molecule; in the identification of certain nucleic acid sequences that contain 5-iodouracil residues and that covalently bind to HIV-1 Rev protein; in the
20   identification of highly specific nucleic acid ligands able to discriminate between the closely related molecules, theophylline and caffeine; as a method to achieve efficient partitioning between oligonucleotides having high and low affinity for a target molecule; and as a method for covalently linking a nucleic
25   acid to its target.

The SELEX method relies on a process of selection and amplification for enrichment of desired candidate positives from a collection of candidates to identify better candidates or the
30   best candidates from the collection. During the selection part of the process from each parent collection, the bulk binding of the populations of candidates becomes increasingly higher as the sequences are amplified, and those sequences unable to interact with the target are eliminated from the population. Hence,

7

"evolution" of the population occurs due to the increased presence due to amplification of candidates which exhibit the desired activity and the effective elimination of undesirable candidates. Amplification is used to increase the presence of
5   desirable products and to separate those products from those that do not react or have a weaker reaction with a target of interest. (WO 96/09316 entitled Parallel Selex)

The Parallel SELEX method describes one potential technique for
10   the identification of DNAs that have facilitating activities as measured by their ability to facilitate formation of a covalent bond between the DNA, including an associated functional unit, and its target. Although this method focuses on the facilitative binding capabilities of DNA, it does not take advantage of the
15   potential for nucleic acids to be evolved in vitro via methods such as Error-prone PCR or Sexual PCR. The method defines the pool, or collection, of DNAs as being evolved due to the enrichment of positives that occurs via an amplification reaction (exponential enrichment). The DNA molecules themselves are never
20   evolved.

SUMMARY OF THE INVENTION

There currently exists need for novel systems which combine the
25   advantages of screening the different types of collections mentioned; one which allows the enrichment by serial selection and facilitates the decoding of the structure of lead candidates afforded by screening nucleotides, and which simultaneously provides the potentially limitless repertoires of diverse
30   molecules for screening offered by chemical compound and natural product collections. The present invention provides a novel approach for creating diverse, complex shape and structure libraries of large or small size agent molecules and for screening said libraries to identify compounds having a wide
35   variety of commercially valuable industrial applications. Not

8

only does the present invention provide a limitless repertoire
of diverse structures that may be screened for biological
activity, but it provides an iterative selection and enhancement
process to define the most active compounds, and it is a process
5   that allows one to solve the structure (if desired) of the most
active compounds rapidly.   These processes for mutating and
selecting compounds to effectively "evolve" chemical groups in
order to identify a most useful compound(s) and the ability to
rapidly solve the structure of identified compounds are
10 significant advantages of the present invention.   These
advantages and other features distinguish the present invention
from previously existing technologies.

The present invention provides a method for identifying one or
15 more complexes from a library of complexes, wherein said complex
or complexes are selected for their ability to perform a
preselected or desired function on a target molecule or by having
a pre-selected structure, each complex being designated a
morphatide, said method comprising: (a) preparing a library of
20 morphatides, comprised of: (i) a scaffolding component selected
from the group consisting of nucleic acid, nucleic acid like
molecule or nucleic acid analog having one or more regions of
randomized sequence; (ii) one or more linker components; and
(iii) one or more agent molecules or type of agent molecules,
25 linked to the scaffolding component by one or more type of linker
components; and (b) screening the library of morphatides prepared
in step (a) by contacting, binding, or associating the
morphatides with one or more suitable target molecules upon which
a morphatide performs a preselected or desired function or to
30 which a morphatide binds or associates through a pre-selected
structure of said morphatide under conditions permitting said
morphatide to perform said preselected or desired function on
said target molecules or permitting said morphatide to bind or
associate with said target molecules through the preselected
35 structure;   (c) separating the morphatides performing the

9

preselected or desired function or binding or associating through
the preselected structure, from the library of morphatides and
target molecules; thereby identifying one or more complexes from
a library of complexes, wherein said complex or complexes are
5   selected for their ability to perform a preselected or desired
function on a target molecule or by having a pre-selected
structure.

The present invention also provides a method for identifying one
10   or more complexes from a library of complexes, wherein said
complex or complexes are selected for their ability to perform
a preselected or desired function on a target molecule or by
having a pre-selected structure, each complex being designated
a morphatide, said method comprising: (a) preparing a library of
15   morphatides, comprised of: (i) a scaffolding component selected
from the group consisting of nucleic acid, nucleic acid like
molecule or nucleic acid analog having one or more regions of
randomized sequence; and (ii) one or more agent molecules or type
of agent molecules, associated, bound, or bonded to the
20   scaffolding component; (b) screening the library of morphatides
prepared in step (a) by contacting, binding, or associating the
morphatides with one or more suitable target molecules upon which
a morphatide performs a preselected or desired function or to
which a morphatide binds or associates through a pre-selected
25   structure of said morphatide under conditions permitting said
morphatide to perform said preselected or desired function on
said target molecules or permitting said morphatide to bind or
associate with said target molecules through the preselected
structure; (c) separating the morphatides performing the
30   preselected or desired function or binding or associating
through the preselected structure, from the library of
morphatides and target molecules; thereby identifying one or more
complexes from a library of complexes, wherein said complex or
complexes are selected for their ability to perform a preselected
35   or desired function on a target molecule or by having a pre-
selected structure.

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The present invention further provides a method of identifying a presence of a substance in a sample from a subject, comprising: (a) obtaining a sample; (b) contacting the sample with one or

5   more types of morphatide identified by either of the methods for identifying one or more complexes so as to form a complex between the morphatide and the substance present in the sample; (c) detecting the complex formed in step (b), thereby identifying the presence of the substance in the sample from the subject.

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This invention further provides a method of diagnosing a subject wherein detection of a complex in step (c) of a method of identifying a presence of a substance in a sample is indicative of a disease.

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Another aspect of the present invention provides a morphatide capable of effectively binding to, crosslinking with, or reacting with multiple types of molecules. Another aspect of the present invention provides a morphatide capable of effectively binding

20   to, crosslinking with, or reacting with one type of molecule.

Still another aspect of the present invention provides a composition comprising the preferred embodiment of the morphatide effective to treat a subject and a pharmaceutically acceptable

25   carrier.

The present invention further provides a morphatide labeled with a detectable marker.

30   This invention also provides a method of treating a subject with compositions of morphatides and morphatides conjugated to therapeutic agents.

In another aspect, this invention provides a method of drug

35   delivery to a target in the body of a subject comprising

11

administration to a subject any of the above-described
compositions of morphatides and morphatides conjugated to
therapeutic agents.

5   This invention further provides a method of drug delivery to a
target in the body of a subject comprising administration to a
subject of the above-described compositions of morphatides and
morphatides conjugated to therapeutic agents, wherein the
morphatide is incapable of being degraded or is slowly degraded
10   after administration to the subject, thereby delivering the
morphatide-bound drug to the target.

This invention still further provides a morphatide that is
capable of binding to any component of an antibody molecule, said
15   antibody having a constant and variable region.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1.   Briefly demonstrates one well known strategy for
20   generating and utilizing Aptamers, a library of
                     nucleotide shapes.

Figures 2A-B.   Present an example of the method described in the
                    present invention beginning with a template
25   nucleic acid molecule as the scaffolding
                    molecule.

Figure 3.   Depicts a similar example of the method described
                    in the present invention, indicating the target
30   molecules bound to a solid support, and the
                    dissociation of the complex molecules from the
                    target molecules occurring via elution with a pH
                    shift.

35   Figure 4.   Depicts the use of a particular type of
                    bioconjugate, phenyl boronic acid, which can be

12

used as a linker in the present method.

DETAILED DESCRIPTION OF THE INVENTION

5   Unlike previously described technologies and methods, the present
     invention is an approach that maintains the facile ability of
     nucleic acids to evolve, while introducing the properties of
     additional components to create molecules with binding properties
     previously restricted to protein-like molecules. The basis of
10   this invention is a Morphatide (previously termed Morphotide).
         A morphatide, as used herein, is a complex comprised of a
     scaffolding component, hereinafter defined, one or more linker
     components, hereinafter defined, and one or more agent molecules,
     hereinafter defined. Once desirable Morphatides are identified,
15   scaffolding components can be separated from the agent molecules,
     evolved to generate a new, different and potentially better
     library of scaffolds, reconnected to the same or different agent
     molecules to generate a new library of Morphatides, and
     rescreened for an even more desirable activity, of either the
20   entire Morphatide or individual components thereof. This
     approach permits the directed evolution of polynucleotide
     molecules which can be disconnected, amplified and evolved. The
     present invention is thus an approach which couples the distinct
     advantage of the self replication of oligonucleotides and their
25   potential to be evolved, with the richness of diversity of
     chemical modifications hitherto associated with other types of
     libraries.

     In accordance with the present invention, libraries comprising
30   structural or scaffolding components, linker molecules, and agent
     molecules are produced. Alternatively, the libraries may
     comprise structural or scaffolding components and agent molecules
     without linker molecules. Useful candidate compounds are
     identified from said shape and structure libraries. The useful
35   candidate compounds can then be separated from the library and
     either modified to generate an even more useful candidate, or

13

utilized directly.

The novel shapes and structures generated utilizing the present invention are named Morphatides. The process of generating and
5   screening a Morphatide library is deemed Morphatide based combinatorial chemistry. Morphatide combinatorial chemistry can begin with a template scaffolding molecule. In one example, if this scaffolding molecule is a nucleic acid molecule, the molecule is amplified utilizing a process known as sloppy PCR,
10 error-prone PCR or mutagenic PCR (PCR Primer, A Laboratory Manual, Cold Spring Harbor Laboratory Press 1995) and nucleotides that have been coupled to, associated with, or attached to a component of a linker molecule or to a linker molecule. The nucleotides can be naturally occurring, novel or unique, or
15 nucleotide analogs. Error-prone, or sloppy or mutagenic, PCR is a process for performing the polymerase chain reaction under conditions where the copying fidelity of the DNA polymerase is low, such that a high rate of point mutations is obtained along the entire length of the PCR product. This process generates
20 various scaffolding molecules with components of linker molecules or linker molecules randomly attached throughout each molecule.
Alternatively, nucleic acid scaffolding molecules (components) can be generated synthetically using techniques well known in the art by sequence randomization of individual nucleic acid bases
25 utilizing naturally occurring nucleotides, novel or unique nucleotides, or nucleotide analogs. (Ecker, D.J. et al., 1993)
Oligonucleotide synthesis is a well characterized chemistry that can allow tight control of the composition of the mixtures of oligonucleotides created. Degenerate sequences can be readily
30 produced. Association to linkers or to linker components can also occur after generation of scaffolding components. A library of Morphatides is then created by associating one or more agent molecules, which in one case have also been coupled or connected to other components of the linker molecule or not, with the
35 scaffolding molecules to connect or associate the components of the linker molecules to each other or to connect or associate the

14

agent molecule to the scaffold/linker mini-complex, thereby generating complexes (Morphatides) assembling the scaffolding molecules, linker molecules and the agent molecules. One can use mixtures of agent molecules attached to one type of

5   linker/scaffold attachment site with the present invention. This library is screened to enrich or select for any desired interaction (typically a binding event) with any target (substrate or substrates) of interest. Novel shape and structure libraries, i.e., Morphatides, identified by the methods of

10   screening provided by the present invention are thereby generated. Subsequent to screening, Morphatides may be utilized directly, scaffolding and/or agent molecules and/or complex molecules may be analyzed and mimics of either the entire Morphatide or components thereof may be created for further use,

15   or components of the Morphatides (agent, linker and scaffolding molecules) may be separated, modified, new Morphatides generated and the entire process repeated (iterative selection process). Once a morphatide or collection of morphatides have been identified via the screening process, the structure of one or

20   more of the complexes (morphatides) can be analyzed to retrieve information in order to allow the generation or creation of chemical or small organic molecules that mimic the selected or desired morphatide.

25   Sequencing techniques can be readily utilized to analyze the scaffolding components of a Morphatide. High-throughput sequencing is well known to the skilled artisan, and the small size of the scaffolding components of Morphatides allows for rapid sequence determination and evaluation of the molecules.

30   It is contemplated herein that other technologies may also be employed to analyze Morphatide molecules. These techniques include utilizing mass spectrometry and nuclear magnetic resonance (NMR) to analyze molecules of interest. Said technologies are well known to the skilled artisan for the

35   evaluation of molecules.

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Alternatively, the components of the selected morphatide or
collection of morphatides can be separated and the scaffolding
component evolved utilizing any of the error prone or sexual PCR
or random or directed mutagenesis techniques well known to one
5   of ordinary skill in the art. This process is demonstrated in
Figures 2(a) and 2(b) and Figure 3.

Interaction of selected Morphatides and target molecules can
occur via binding, contact, connection or other association
10 between the entire complex (Morphatide) and the target, or
between any portion of the Morphatide and the target. Each
component of the Morphatide can contribute to the shape,
structure and/or function of the Morphatide, however actual
interaction with a target can occur between any one or more of
15 the components and the target. For instance, any individual
component, such as the scaffolding component, linker component,
or agent molecule can be the site for binding or associating with
the target, or any combination thereof, such as the scaffolding
component and the linker component, or the scaffolding component
20 and the agent molecule, etc. can contribute to the binding or
association. After desirable Morphatides are identified using
the method described, one can further select even more desirable
Morphatides by utilizing sexual PCR to effectively eliminate
portions of selected Morphatides that do not contribute to the
25 binding or interaction with the target. Sexual PCR, also known
as DNA shuffling, (U.S. Patent No. 5,605,793, entitled "Methods
for In-vitro Recombination", Feb. 25, 1997) is employed utilizing
modified scaffolding components of the selected Morphatides and
similar non-modified scaffolding components to generate further
30 optimized Morphatides. In this process, scaffolding components
with attached linkers or linker components of previously
identified Morphatides are first separated from the agent
molecules and combined with scaffolding components having the
same nucleic acid sequence, however not being associated with
35 linkers or linker components. The DNA is then fragmented and

16

reassembled using the sexual PCR technique to generate new scaffolding molecules (components) which are very similar to the original scaffolding molecules, however different. Certain linker attachment sites in the newly generated scaffolding

5   molecules may have been eliminated. Reattachment or association of agent molecules to generate another set of Morphatides can yield a further optimized Morphatide or Morphatides.

In Figures 2(a) and 2(b), an example of the process begins with

10 a template nucleic acid molecule. The template is amplified in the presence of nucleotides which have been previously associated with linker components ("coupling nucleotides"), generating scaffolding molecules. The amplification is performed under conditions to allow random incorporation of the coupling

15 nucleotides. Morphatides are created by, in this example, binding the scaffolding molecules to agent molecules, in this example, chemicals. As used herein, chemicals, include but are not limited to molecules with aliphatic, aromatic, carboxyl, hydroxyl or amine groups. Binding, or desired Morphatides, are

20 selected for by binding to a substrate of interest, and a wash step is used to remove all non-binding substrates and/or Morphatides. In this example, the substrate is then separated from the Morphatide, the chemical group is released. The enriched or selected for scaffolding molecule(s) are then

25 subjected to amplification under conditions which allow for random incorporation of coupling nucleotides, again, and the process continues as before until the desired Morphatide or collection of Morphatides is finally determined or recovered.

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Figure 3 depicts the process in a slightly different format. In this example, the target (substrate) has also been immobilized to a solid surface, and the target (substrate) is separated from the Morphatide after the first screen by elution with a pH shift.

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Variable scaffolding molecules can be generated or further
modified utilizing a variety of techniques known in the art,
including "sloppy", "error-prone" or "mutagenic" PCR, as
5   mentioned. Another technique which may be employed is known as
"sexual PCR". Sexual PCR is the forced homologous recombination
between nucleic acid molecules of different but highly related
sequences in vitro, caused by random fragmentation of the nucleic
acid molecules, priming of the fragments on a non-parental
10   nucleic acid molecule based on sequence homology, followed by
fixation of the crossover by primer extensions in an
amplification reaction. Sexual PCR is used for the in vitro
evolution of DNA sequences. The libraries of recombinants that
are created by sexual PCR are selected in vitro or in vivo for
15   the best combinations of mutations at the nucleic acid, protein
or metabolite level. The process of recombination, selection and
amplification can be repeated for as many cycles as necessary to
identify the best combinations. Once a collection of Morphatides
has been enriched, components of the molecules can be separated
20   from each other, and sexual PCR can be performed to create new
scaffolding molecules. After reassociation, the newly generated
Morphatides can be further enriched or screened for activity of
interest.

25   Scaffolding molecules can also be generated or further modified
by other mutagenic techniques, such as cassette mutagenesis or
site directed mutagenesis. Cassette mutagenesis is any process
for replacing a small region of a double stranded DNA molecule
with a synthetic oligonucleotide "cassette" that differs from the
30   native sequence. The oligonucleotide often contains completely
and/or partially randomized native sequence.

It is further contemplated herein that amplification of
scaffolding components, or scaffolding components associated with
35   linker components, or Morphatides as a whole, can be achieved in

vivo in any aspect of the method of the present invention wherein amplification is beneficial. In _vivo_ amplification can be performed, by example, by utilizing Topoisomerase cloning (Invitrogen Corp.).

5   Scaffolding components of the present invention are molecules that contribute to the conformational diversity and contribute to the complexity of the morphatide libraries to be screened. Diverse scaffolding molecules allow each complex molecule in the library to have a distinct shape. "Shape" has been defined as

10   "the net sum of all the molecular properties and dynamic features that would affect interaction (of molecules) with other molecules". (Kenan,D.J. et al. 1994) Nucleic acid scaffolding components of the present invention can also provide the capability to clone, amplify, and evolve components of the

15   morphatides and to decode the structure of the selected morphatides.

Nucleic acid scaffolding molecules can consist of single stranded, double stranded, triple stranded or branched DNA or RNA

20   molecules. DNA or RNA scaffolding molecules can be generated using synthetic or biosynthetic methods. Nucleotide analogs to nucleotide bases, such as 5-azo-cytodine, inosine or 7-deaza-guanine can be employed to increase the complexity of the resulting scaffolding molecules. Nucleotide bases may be

25   modified prior to, or after, generation of nucleic acid scaffolding molecules. Nucleotide scaffolding molecules can be generated by randomization of the order of individual bases or modified bases. (Houghten, 1985; Beaudry and Joyce, 1992) Such techniques are well known to those skilled in the art.

30
Scaffolding molecules of the present invention can comprise a variable core flanked by short sequences to facilitate amplification. The variable core can be designed such that the sites of connection to the linker can be readily identified.

35   This could facilitate the rapid determination of the structure

19

of an optimized scaffolding molecule component of a Morphatide.
For example, by constructing the variable core as follows, this
challenge can be resolved: since a polynucleotide comprising
just 3 of the 4 normal bases provides adequate variation for the

5   scaffolding molecule, the remaining base can be used to serve as
the connector or attachment site for the linker to carry the
agent molecule. By having one base function as the attachment
site for the linker in the construction of the variable core, the
number of linker sites can be controlled and the position readily

10   ascertained.

Alternatively, any one or more of the different bases or any base
mimic can be used as an attachment site. Preferably, sites of
connection of the linker and agent are internal in the

15   scaffolding component to potentially provide greater shape and
structural diversity. Connection sites restricted to terminal
sites in the scaffolding components may limit this desired
feature. It should be noted that if one were only utilizing a
modified nucleotide to interact or bind to a target of interest,

20   internal connection or modified sites could severely interfere
with any further hybridization event of this nucleotide. With
the present invention, however, the greatest shape and structural
diversity can be provided by utilizing internal sites of the
scaffolding molecules for connection.

25

It is recognized that novel, or unique bases or base analogs, not
included in the 4 normal bases, can be employed in the present
invention.   Such novel or unique bases or base analogs can
include, but are not limited to, bases, base analogs or "mimics"

30   such as difluorotoluene deoxynucleosides,(Rawls, R., 1997) or
other molecules which can be incorporated by any nucleic acid
polymerase to any degree, and/or which do or do not effect
hybridization, binding or association of a nucleotide to any
other molecule or molecules.

35

It is also recognized that more than one nucleic acid polymerase can be employed simultaneously to incorporate bases or base analogs and/or "linker" attached bases or base analogs.

5   Scaffolding molecules can also be modified post generation via a variety of chemical techniques, such as that employed in the Maxam and Gilbert DNA sequencing procedure, or via the utilization of known mutagens such as UV light, or DNA binding proteins which can cause modifications to specific or random
10 nucleotides. The Maxam and Gilbert DNA sequencing procedure is very well known in the art. (Maxam, A.M. and Gilbert, W. 1980) The procedure involves the treatment of DNA samples with a chemical that specifically damages or modifies one or two of the four bases in DNA in a controlled reaction where only a few of
15 the sites are nicked in any one DNA molecule. The chemicals utilized in this procedure can be utilized in the present invention. Mutatable nucleotides, such as methylated cytosines may be utilized to generate scaffolding molecules. Mutagens such as (+)-CC-1065; (+)-CC-1065(N3-Adenine); trivalent chromium or
20 trivalent chromium salts; polycyclic aromatic hydro-carbons (PAH), such as 7-bromomethyl-benz[a]anthracene (BMA); Tris(2,3-dibromo-propyl)phosphate (Tris-BP); 1,2-dibromo-3-chloro-propane ("DBCP"); 2-bromoacrolein (2BA); benzo[a]pyrene-7,8-dihydrodiol-9-10-3epoxide (BPDE); platinum(II) halogen salts; N-hydroxy-2-
25 amino-3-methyl-imidazo[4,5-f]-quinoline (N-hydroxy-]Q); or N-hydroxy-2-amino-1-methyl-6-phenylimidazo[4,5-f]-pyridine   (N-hydroxy-Ph]P) can be added to modify nucleic acids by adding chemical adducts to specific or random nucleotides. These adducts or adducted sites may then be utilized as linkers to
30 which agent molecules, hereinafter defined, can be attached, or can themselves act as agent molecules.

Agent molecules of the present invention are molecules that further provide for complex and diverse morphatides. As used

herein, an agent molecule is a molecule that can be recognized by or can recognize a particular target molecule or portion of a target molecule of interest. This recognition is typically a binding event or some other event of association. Screening of

5   and selection from shape and structure libraries (Morphatides) represents an approach to generating complexes of molecules that recognize and bind target molecules. The molecules that recognize and bind target molecules may be the entire complex of molecules (the Morphatides) or individual components thereof,

10   including but not limited to the agent molecules or the scaffolding components.

Agent molecules can consist of natural products such as natural polymers (peptides, oligonucleotides, etc.) or natural

15   nonpolymeric molecules (antibodies, etc.), or of unnatural products such as synthetic polymers or other synthesized nonpolymeric molecules. Examples of agent molecules include, but are not limited to, peptides, nucleic acids, carbohydrates, proteins, and other molecules synthetic compounds, agonists and

20   antagonists for cell receptors, hormones, chemicals, chemical structures, sugars, cofactors, enzymes and other proteins, enzyme substrates, and drugs. There are virtually an unlimited number of agent molecules that may be screened using the present invention. Assembly of agent molecules can occur by systematic

25   association of building block components of agent molecules using chemical, biological, or biosynthetic procedures.

Synthesis methods are well known in the art. Building block components of agent molecules can be diverse and fairly complex.

30   Assembly of such building blocks could yield a broad, diverse collection of agent molecules providing diverse physicochemical properties, functionality, charge, and conformation. Building blocks could have groups with high reactive functionality that allow for multiple new covalent combinations and many potential

35   connecting permutations providing diverse, spatial relationships (carbohydrates, for example, where almost every carbon in a given

molecule has a hydroxyl or other oxygen-containing functional group attached to it).

5   Linker molecules of the present invention are molecules that can allow connection or association of the scaffolding molecules to agent molecules. Linker molecules can consist of chemical compounds. Typically, the chemical compounds contain one or more reactive groups, allowing the linkers to associate and preferentially to be cleaved or disconnected by means of a
10   specific reaction or reaction steps. Linkers also have appropriate functional groups at each end for coupling to the scaffolding molecules and to the agent molecules. Preferred linkers are those whose cleavage or disconnection is controllable. Particularly preferred linkers are those whose
15   cleavage or disconnection is reversible. Illustrative examples of suitable linkers include bioconjugates such as Phenylboronic Acid, DNA binding proteins, or biotin/streptavidin. In addition to the cleavable groups, suitable linkers may contain other groups that influence or do not influence the cleavage reaction,
20   which are suitable for enriching or separating scaffolding molecules from agent molecules.

Cleavable linkers may also consist of a cleavable component and a constant component, which is the same for either all
25   scaffolding molecules or for all agent molecules. The constant part may consist of chemical compounds which permit attachment to both the cleavable part of the linker and to other chemical groups or to other molecules. An example of a constant component is an invariable part of the scaffolding molecule or of the agent
30   molecules.

Nucleotide molecules can be used alone as scaffolding molecules, or linker molecules can be employed in the present invention to force the shape of the resulting complex molecules to yield novel

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shapes, or scaffolding molecules.

There are a variety of linkers that may be useful for purposes
of the present invention. For instance, linker molecules could
5   be based upon the phenylboronic acid complexing moieties
(Yurkevich 1969). Phenylboronic acids are known to interact with
a wide range of polar molecules having the requisite
functionalities (Middle 1983; Frantzen 1995). Phenylboronic
acid, like boric acid, is a Lewis acid, and ionizes not by direct
10   deprotonation, but by hydration to yield the tetrahedral
phenylboronate anion (pKa=8.86). A variety of phenylboronic acid
molecules with varying pKa's are commercially available.
Molecular variations can also be generated. Ionization is
fundamental for complexation causing a change from trigonal
15   coordination to tetrahedral coordination. Bioconjugation with
phenylboronic acid molecules has been achieved between compounds
having diol functionalities (e.g. carbohydrates) to immobilized
phenylboronate anion to form cyclic esters under alkaline
conditions. Release is effected by pH shifts. Phenylboronic
20   acid modified dUTP linker molecules have also been incorporated
into oligomers using DNA polymerases as an alternative to DNA
labeling and purification via biotin incorporation.
Bioconjugation via linkers such as the phenylboronic acid linker
can simplify the reversibility of the coupling reaction, enabling
25   attachment of agent groups that cannot generally be incorporated
by DNA polymerases. In addition, the phenylboronic acid molecule
causes minimal interference with respect to DNA hybridization and
base incorporation with a deoxynucleotide triphosphate attached
to it.
30

Phenylboronic acid bioconjugate complexes are suitable for use
as linker molecules in the present invention. It is a preferred
embodiment. Methods for associating and dissociating suitable
linkers to many different types of potential molecules, such as

the agent molecules and/or scaffolding molecules referred to in the present invention are known to one of ordinary skill in the art. (WO 95/20591) These methods include but are not limited to those described in WO 95/20591, as well as those using biotin-
5   streptavidin. Figure 4 depicts the use of this type of candidate bioconjugate, indicating the fact that standard chemistry can be used to attach one component of the bioconjugate (linker) to candidate scaffolding molecules (Binder 1 or 2), and to attach another component of the linker to candidate agent molecules
10   (Binder 2 or 1, depending on which Binder scaffolding molecule is). A condensation reaction then associates the linker components, creating a complex, or Morphatide.

Disulfide based coupling systems can also be utilized as linkers
15   in the method of the present invention. Disulfide based coupling systems, such as that described herein, offer the benefit of being reversible under mild redox conditions. The system is selective for thiol groups and nucleic acid is stable under the conditions in which the system functions.
20
In the system, depicted below, the nucleoside triphosphate can be linked by an amine containing linker arm attached to the nucleoside base component of a scaffolding molecule. An agent molecule can then be linked by a thiol containing linker arm.
25
Heterobifunctional cross-linkers, such as N-succinimidyl 3-[pyridyldithio]propionate (SPDP) (described by Hermanson, Greg T., Bioconjugate Techniques, Academic, San Diego, CA 1995, p.230)(available from Pierce), which link amino containing
30   molecules to a thiol containing molecule can be employed. The heterobifunctional cross-linker SPDP is available from Pierce as are other related crosslinkers with different chain lengths making this a versatile system for manipulation of the distance between the DNA oligomer and the chemical group of interest.
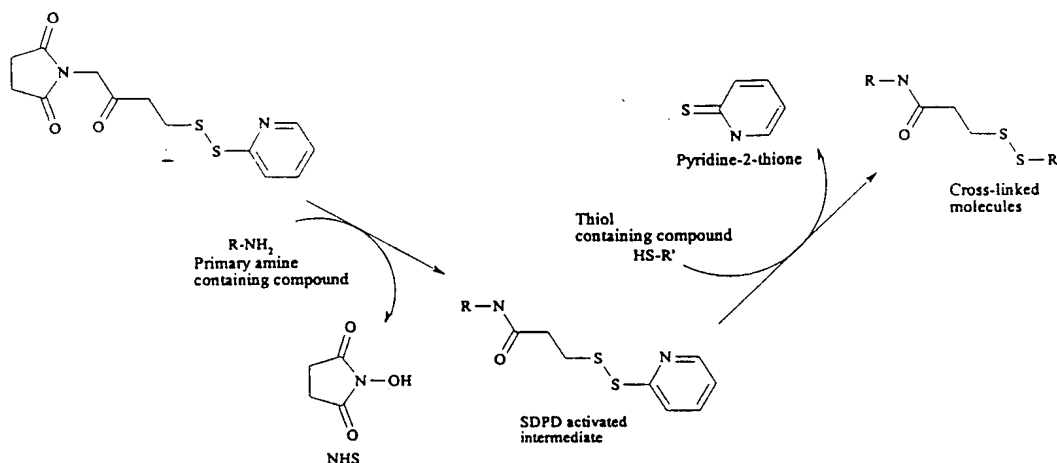
The 5-position of cytidine and uridine, and the 8-position of
adenine and guanine are preferable positions for association with
5   the linker.   It is contemplated that other positions can be
utilized.   Alternatively, a
diazirine coupling system could be used to incorporate the linker
into a modified nucleotide containing a thiol group through the
maleimide portion and the chemical group (CG) through the
aryldiaziridine portion.   However, the system is less selective
15   and less reproducible than the disulfide coupling system.

It is contemplated that modified nucleotide molecules, such as
pseudouridine (Ψ) or other molecules which can be utilized to
generate polymers similar to nucleic acid, but which are more
20   stable than nucleic acids, such as PNA monomers, can also be
utilized with the methods of the present invention.

Nucleotides associated with linker molecules are incorporated
into scaffolding components.   Morphatides are generated on
25   treatment of the scaffolding components with agent molecules
derivatized to contain thiols.

26

As used herein a binder is any molecule which is to be attached to another molecule by the linker. Binders include but are not limited to nucleic acids, amino acids and chemical groups.

5   Therefore, the components of a Morphatide, i.e., the scaffolding component and the agent molecules are binders.

Linkers can be coupled via functional groups to one or more different sites on both scaffold molecules and agent molecules.

10   Synthesis methods to attach phenylboronic acid to other molecules are known (WO 95/20591). Connecting scaffold molecules to agent molecules via a reversibly connectable linker such as phenylboronic acid yields conformationally diverse library complex molecules.

15

Target molecules of the present invention are molecules to which morphatides are selected to bind, associate, or interact. Target molecules can be any molecule of interest. Examples of such molecules include, but are not limited to, cell membrane

20   receptors, antibodies, lectins, polysaccharides, cells, cellular membranes, organelles, and chemicals. Preferably, target molecules of interest are bound to a support. Examples of such supports include, but are not limited to, solid surfaces, beads, particles, or other support.

25

Libraries of complexes (morphatides) can be screened for any target molecule of interest, any chemical activity such as catalysis of inorganic and organic reactions, or any biological activity of interest which may be known in the art. Biological

30   activities known in the art include, but are not limited to, antimicrobial activities, antitumor activities, enzyme inhibiting activities, receptor binding activities, growth promotion activities, antibody binding activities and biofilms. Many screening assays are available and known for these activities and

35   a variety of other biological responses, and any can be used with

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the present invention.

Evolution via such techniques as previously described, such as
Sexual PCR, may be performed on undesirable scaffolding molecules
5   subsequent to identification and separation from desirable
complexes to eventually create new collections of complexes which
can be rescreened for desirable activity.- Undesirable
scaffolding molecules are those scaffolding molecules that are
part of the Morphatides that do not bind or pass the screening
10   test.

A variety of screening techniques are known in the art and can
be used in the present invention. (Mullinax, R., et. al., 1990;
Barbas, C., et. al., 1991; Castagnoli, L., et. al., 1991;
15   Garrard, L., et. al., 1991; McCafferty, J., et. al., 1990;
Clackson, T., et. al., 1991; Kang, A., et. al., 1991; Hoogenboom,
H., et. al., 1991; Chang, C., et. al., 1991;)

It is recognized that with any screening technique involving a
20   binding event, steps may be taken to decrease potential non-
specific binding of Morphatides. These steps are well known in
the art, and include but are not limited to enriching, isolating
or separating bound or otherwise associated complexes from
unbound or unassociated complexes can occur via a variety of
25   enrichment, isolation and separation techniques well known in the
art. Typical enrichment, isolation and separation techniques
involve solvent partitioning and/or conventional chromatography.

Enriching, isolating or separating bound complexes (morphatides)
30   from target molecules can occur via a variety of isolation and
separation techniques, also well known in the art, and depend on
the nature of the connection between the complex and the target
molecule.   Typical enrichment, isolation and separation
strategies involve elution or digestion steps.
35

More particularly, the present invention provides a method for

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identifying one or more complexes from a library of complexes,
wherein said complex or complexes are selected for their ability
to perform a preselected or desired function on a target molecule
or by having a pre-selected structure, each complex being
5   designated a morphatide, said method comprising: (a) preparing
a library of morphatides, comprised of: (i) a scaffolding
component selected from the group consisting of nucleic acid,
nucleic acid like molecule or nucleic acid analog having one or
more regions of randomized sequence; (ii) one or more linker
10   components; and (iii) one or more agent molecules or type of
agent molecules, linked to the scaffolding component by one or
more type of linker components; and (b) screening the library of
morphatides prepared in step (a) by contacting, binding, or
associating the morphatides with one or more suitable target
15   molecules upon which a morphatide performs a preselected or
desired function or to which a morphatide binds or associates
through a pre-selected structure of said morphatide under
conditions permitting said morphatide to perform said preselected
or desired function on said target molecules or permitting said
20   morphatide to bind or associate with said target molecules
through the preselected structure; (c) separating the morphatides
performing the preselected or desired function or binding or
associating through the preselected structure, from the library
of morphatides and target molecules; thereby identifying one or
25   more complexes from a library of complexes, wherein said complex
or complexes are selected for their ability to perform a
preselected or desired function on a target molecule or by having
a pre-selected structure.

30   Another aspect of the present invention provides a method for
identifying one or more complexes from a library of complexes,
wherein said complex or complexes are selected for their ability
to perform a preselected or desired function on a target molecule
or by having a pre-selected structure, each complex being
35   designated a morphatide, said method comprising: (a) preparing
a library of morphatides, comprised of: (i) a scaffolding

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component selected from the group consisting of nucleic acid, nucleic acid like molecule or nucleic acid analog having one or more regions of randomized sequence; and (ii) one or more agent molecules or type of agent molecules, associated, bound, or

5   bonded to the scaffolding component; (b) screening the library of morphatides prepared in step (a) by contacting, binding, or associating the morphatides with one or more suitable target molecules upon which a morphatide performs a preselected or desired function or to which a morphatide binds or associates

10   through a pre-selected structure of said morphatide under conditions permitting said morphatide to perform said preselected or desired function on said target molecules or permitting said morphatide to bind or associate with said target molecules through the preselected structure; (c) separating the morphatides

15   performing the preselected or desired function or binding or associating through the preselected structure, from the library of morphatides and target molecules; thereby identifying one or more complexes from a library of complexes, wherein said complex or complexes are selected for their ability to perform a

20   preselected or desired function on a target molecule or by having a pre-selected structure.

The contacting, binding, or associating of the morphatides to target molecule(s) or of a morphatide conjugated to a therapeutic

25   agent(s), as described infra, may be through ionic, covalent, hydrophobic or hydrogen bonds, or through Van der Waals forces.

In an embodiment of the above-described methods, the separation of step (c) is performed by either (a) separating the morphatides

30   which do not perform the preselected or desired function or which do not bind or associate through a pre-selected structure or (b) separating the morphatides which perform the preselected or desired function or which bind or associate through a pre-selected structure.

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Methods of separating are well known to one of ordinary skill in

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the art and include but are not limited to centrifugation;
electrophoresis; biopanning; solubility differences;
chromatography; fluorescence sorting, properties such as
physical, chemical or electrical; photochemical; magnetic; and
5 visible detection.

In a preferred embodiment of the above-described methods said
target molecule is bound to a solid support. In a preferred
embodiment the preselected or desired function performed by the
10 complex (es) on a target molecule is selected from the group
consisting of binding to or associating with said target
molecule; reacting with said target molecule and changing the
property of said target molecule; having an affinity for and
binding to a specific ligand; performance of a biological
15 activity, wherein said biological activity is selected from the
group consisting of antimicrobial activity, antitumor activity,
enzyme inhibiting activity, enzyme enhancing activity, receptor
binding activity, growth promotion activity, antibody binding
activity; formation of a biofilm; enzymatic activity, immune
20 modulating activity, cell signaling activity, polymerizing
activity, and encapsulating activity.

In another embodiment the contacting, binding or association is
selected from the group consisting of multiple complexes acting
25 on a single target molecule, a single complex acting on multiple
target molecules, components of one or more complexes acting on
multiple target molecules, components of one or more complexes
acting on a single target molecule, and multiple complexes
acting on multiple target molecules.
30
In a preferred embodiment the scaffolding component comprises
naturally occurring nucleotides, novel or unique bases, base
analogs or any combination thereof. In an embodiment said
scaffolding component comprises synthetically generated nucleic
35 acids, nucleic acid like molecules, nucleic acid analogs. In an

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embodiment the nucleic acid like molecule is a difluorotoluene or related deoxynucleoside.

In another embodiment of the above-described methods said
5   scaffolding component consists of subunits which are capable of being incorporated by one or more nucleic acid polymerases or reverse transcriptases and which can, when polymerized, generate hybridizable polymers with hydrogen bonding or hybridizable polymers without hydrogen bonding. In another embodiment said
10   scaffolding component comprises nucleic acids having regions of conserved sequences and one or more regions of randomized sequences. In a preferred embodiment the scaffolding component(s) are comprised of two fixed regions of nucleotides and one region of randomized nucleotides between the two fixed regions. In an
15   embodiment the linker component is associated to a base of the scaffolding component either before or after the scaffolding component is made. In another embodiment the randomized region is comprised of: (a) three of the four bases occurring with similar frequency; and (b) one of the four bases occurring at a
20   rare frequency. In an embodiment one of the bases occurring with similar frequency is associated with or binds with the linker component. In an embodiment one of the four bases occurring at a rare frequency is associated with or binds with the linker component. In an embodiment the position of the base with the
25   linker attached is determined by nucleotide sequencing or mass spectrophotometry.

In another embodiment each scaffolding component comprises more than one different nucleic acid base being attached to a linker
30   component, said nucleic acid base being incorporated into the scaffolding component either during PCR amplification or during synthesis of the nucleic acids. In an embodiment the incorporated nucleic acid base to which the linker component is attached is a rare base. In an embodiment the base to which the linker is
35   attached is modified, said modification being by chemical

reaction either before or after incorporation during PCR amplification or during synthesis of the nucleic acids.

5   In further embodiment the incorporated nucleic acid base to which the linker component is attached is a different modified base, said base being any of the four bases or analogs of said bases, said base being modified by a reaction. In a preferred embodiment the incorporated base to which the linker component is attached is located internally in the scaffolding component.

10

In a further preferred embodiment one or more of said linker components are reversible. In an embodiment one or more of said linker components are non-reversible. In an embodiment one or more of said linker components cannot be amplified (any kind of
15   mathematical increase in the number of molecules) in vitro or in vivo. In another embodiment one or more of said scaffolding components associated with one or more of said linker components is amplifiable in vitro or in vivo. In an embodiment said one or more of said linker components associated with one or more of
20   said agent molecules cannot be amplified in vitro or in vivo. In a preferred embodiment the entire morphatide is amplifiable. In a further preferred embodiment one or more of said linker components comprise reversibly connectable components or parts.

25   In an embodiment a first linker component either reversibly or non-reversibly associated with a scaffolding component and a second linker component either reversibly or non-reversibly associated with an agent molecule are connected together to generate a scaffolding component linked to an agent molecule by
30   the connectable first and second components of said linker component. In a preferred embodiment the linker component is selected from the group consisting of a phenyl-boronic acid linker, a thio linker, and a biotin-streptavidin linker. In an embodiment the thio linker is cysteine. In another embodiment
35   the scaffolding component is associated to one or more agent

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molecules, wherein said agent molecule is a peptide by a peptide bond. In an embodiment the linker component is selected from the group consisting of a nucleic acid binding protein and a chelating molecule. In an embodiment the linker component is
5   bound covalently to either the scaffolding component or to the agent molecule. In another embodiment the linker component is bound noncovalently to either the scaffolding component or to the agent molecule. In an embodiment of the above-described methods said agent molecules are selected from the group consisting of
10   naturally occurring polymers, synthetically generated polymers, and non-polymeric molecules.

In another embodiment of the above-described methods the library of complexes is prepared by: (a) coupling the linker molecules
15   or components of the linker molecules to either the scaffolding components, to form scaffolding component-linker molecules or to the agent molecules, to form agent molecule-linker molecules; and (b) generating a linkage between the scaffolding component-linker molecules and the agent molecules or between the scaffolding
20   components and the agent molecule-linker molecules to yield the complexes, thereby preparing a library of complexes. In an embodiment said scaffolding components are prepared for coupling to linker molecules via chemical reaction yielding modified nucleotides. In a further embodiment said chemical reaction
25   involves treating the scaffolding components with one or more mutagens to add one or more base specific or non-specific adduct(s), resulting in adducted scaffolding molecules, that enable increased reactivity of the base to the linker or directly to the agent molecules. In a still further embodiment said
30   chemical reaction involves treating the scaffolding components with one or more mutagens to add one or more base specific or non-specific adduct(s), resulting in adducted scaffolding molecules, said adduct acting as either a linker or an agent molecule. In another embodiment the adducted scaffolding
35   components are amplifiable. In a preferred embodiment said

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mutagen is UV light, any other nucleic acid mutagen, or a nucleic
acid binding protein. In a further preferred embodiment said
chemical reaction involves treating scaffolding components with
Maxam & Gilbert based chemistries to generate increased
5   reactivity _of one or more bases to a linker or to an agent
molecules.

In another embodiment said scaffolding components are prepared
for coupling to the linker molecules via a non-chemical reaction
10 yielding modified nucleotides. In an embodiment said non-chemical
reaction is an enzymatic reaction.

In further embodiment of the above-described methods said methods
further comprise after step (b): (a) disassociating the
15 scaffolding component of the complex performing the preselected
or desired function from the agent molecule or molecules; (b)
generating modified scaffolding components; (c) associating the
different scaffolding molecules with agent molecules to generate
different morphatides; (d) rescreening the different morphatides
20 by repeating steps (b) and (c) of claims 1 or 2 to identify new
desired candidate morphatides.

In an embodiment said modification of the scaffolding components
occurs via a random or directed mutagenesis technique. In an
25 embodiment said random or directed mutagenesis techniques are
selected from the group consisting of error-prone PCR or sexual
PCR by performing a suitable number of cycles on the scaffolding
components, resulting in one or more base changes in some
percentage of the scaffolding components; cassette mutagenesis;
30 and site directed mutagenesis. Such techniques are well known
to one of ordinary skill in the art. One example of a random
mutagenesis technique is termed "PCR mutagenesis" (PCR Primer,
A Laboratory Manual, Cold Spring Harbor Laboratory Press 1995).
Another mutagenesis technique, previously mentioned, is termed
35 DNA shuffling or Sexual PCR (W. P. C. Stemmer, 1994). Another

mutagenesis technique known to one of ordinary skill in the art
is Combinatorial Multiple Cassette Mutagenesis [Biotechniques,
(1995)]. In vitro evolution procedures of nucleic acids have
also been described by several other groups over the last many
5   years(A. Beaudry and G. Joyce).

Another technique, hereinafter referred to as breeding, may be
used. Breeding is similar to the process of shuffling without
the fragmentation step. Shuffling, as known in the art, begins
10   with a collection of similar sequences (~80% homology) typically
genes encoding molecules of interest. The sequences are treated
with DNase to fragment the nucleic acid, and amplification takes
place with the fragments. The nucleic acid sequences in a
Morphatide are relatively short fragments (hundreds of bases),
15   compared to the size of most genes (thousand(s) of bases). With
breeding,   evolution   of   a   Morphatide,   or   collection   of
Morphatides, can take place without the fragmentation step
normally associated with the shuffling process. Crossover
amplification   (PCR)   of   the   sequences   of   the   scaffolding
20   components may be performed to achieve breeding of the molecules.
The example provided herein details breeding fragments, utilizing
a hybridization/extension/ amplification reaction (a process
similar to cross-over PCR).

25   Scaffolding component(s) from a Morphatide or collection of
Morphatides can also be modified in vivo utilizing host organisms
deficient in mismatch repair systems.

One can rely on homologous recombination between areas of
30   homology present in the scaffolding component of the Morphatides
in   an   enriched   or   unenriched   collection.   Homologous
recombination can be employed in an in vivo strategy of breeding
molecules. The term "homologous" means that one single-stranded
nucleic acid sequence may hybridize to a complementary single-
35   stranded nucleic acid sequence. The degree of hybridization may
depend on a number of factors including the amount of identity
between the sequences and the hybridization conditions such as

temperature and salt concentration. Preferably the region of identity is greater than about 5 base pairs. Homologous recombination is well known to the skilled artisan.

5   Scaffolding components from selected or unselected Morphatides are rendered double stranded, ligated into a vector, and transformed into a host organism, utilizing techniques well known in the art. Sequences of homology present in the sequences of the scaffolding components result in recombination of the
10   sequences, generating new molecules which can be recovered using techniques known to the skilled artisan. The recovered scaffolding components can be utilized to generate new Morphatide molecules and screened for an activity of interest utilizing techniques described herein.

15   Any vector which will propagate in a host organism of choice may be utilized. Vectors allowing multiple scaffolding components to be cloned into a single vector are preferable. For example, "polycos" vectors (Alting-Mees M.A. and Short J.M., Gene, 1993 Dec 27, 137:1, 93-100) can be utilized. Any other vector,
20   including M13 and phagemid cloning vectors, can also be utilized, and final constructs can be co-transfected into the host organism of choice. Systems for cloning PCR fragments can be utilized. For example, the TA Cloning system available from Invitrogen Corporation in Carlsbad, CA, or the PCR-Script Cloning system
25   available from Stratagene Cloning Systems may be employed. Multiple types of vectors can also be utilized as long as their origins of replication are compatible in the host. Cloning vehicles containing scaffolding components are used to transform a host cell. Such host cell preferably is deficient in mismatch
30   repair systems. Said host cells are well known to the skilled artisan. For example, XL1-Red cells are mismatch repair deficient and are commercially available (Stratagene Cloning Systems, La Jolla, CA).

35   It is contemplated that the above described process of homologous recombination could be performed in vitro, if one were to include in the reaction necessary reaction components to allow homologous

recombination between two or more nucleic acid strands.

Once two or more Morphatides of interest have been identified,
scaffolding component sequences may be analyzed, for instance by
sequencing the scaffolding components, and utilized to generate
smaller sequences, such as 30mer sequences, which represent
regions of the scaffold sequence. These smaller sequences may
be generated synthetically or biologically utilizing techniques
well known in the art. Said smaller sequences may be employed
in a shuffling reaction to generate novel scaffolding molecules.
Hence, rather than fragmenting the sequences as is performed in
standard shuffling, known oligonucleotide sequences are generated
and "bred." It is contemplated that oligonucleotide sequences
can also be employed to generate new longer sequences utilizing
ligase to build longer molecules, versus polymerase to extend in
a crossover amplification reaction. The following diagram
depicts this concept:

Scaffolding components:

synthesize fragments

randomly ligate
fragments
(i.e. RNA ligase)

breed | (utilizing a polymerase)
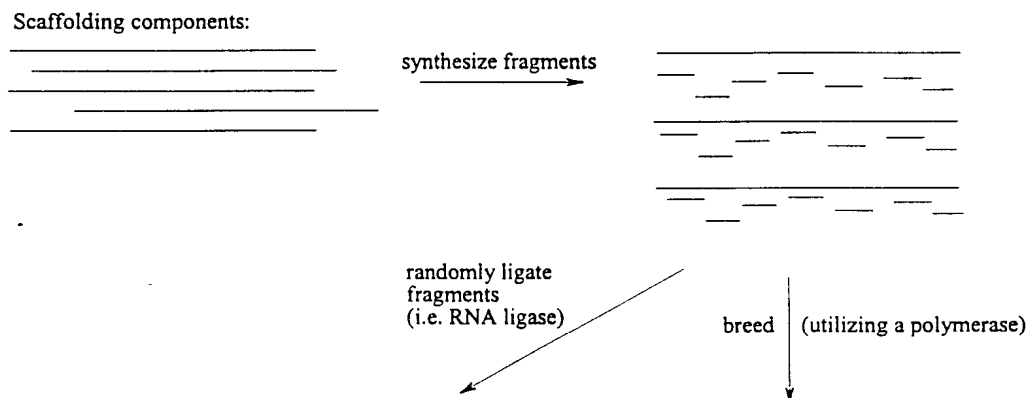
It is contemplated that evolution of Morphatide molecules can
occur between scaffolding components of Morphatides, scaffolding
components associated with linker moieties, or Morphatide
molecules as a whole. Where scaffolding components or
scaffolding components associated with linker moieties are bred,
linkers and/or agent molecules can be associated subsequent to
the reaction. Morphatide molecules which can be evolved as a
whole do not need to be dissociated into smaller components prior

to the evolution process.

Once Morphatides have been evolved, and enriched or selected for optimal candidate molecules, a process of "deconvolution" may be
5   performed to find even more optimal molecules. The evolution process of the method of the present invention allows one to significantly increase the complexity (diversity) of the sample to be screened. The best molecules for the desired activity are selected from the most diverse sample possible. Subsequent to
10 evolution and screening, the complexity of the newly identified Morphatides may be decreased to find an even further optimal activity of interest. "Deconvolution" is a process whereby any non-essential positions of linkers/agent molecules associated with the scaffolding components can be eliminated. The process
15 of deconvolution allows one to select against these non-essential positions. For example, molecule (A) may be determined to possess a desirable activity.
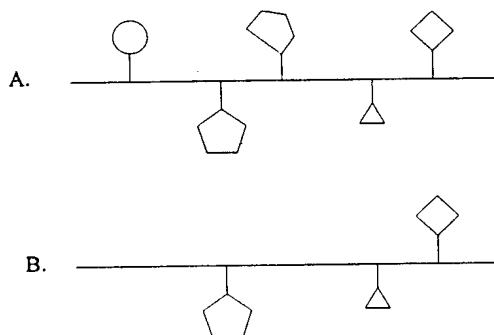
However, molecule (B) may possess optimal activity, but may not
20 have been present in the original diverse sample. The process of "deconvolution" allows one to identify (B) from a new collection of Morphatides consisting of every combination of linker/agent association sites represented in a Morphatide. For example, Morphatide (A) above has 5 sites of association of
25 linker/agent molecules. The new collection generated via the process of deconvolution would have molecules containing only the first, second, third, fourth, or fifth sites, the first and second, first and third, first and fourth, or first and fifth

39

sites, the second and third, second and fourth, second and fifth
sites, etc. Thus from this collection, one can identify an
optimal molecule.

5   The process of deconvolution can be performed by first generating
oligonucleotides representing sequence of the scaffolding
components of identified Morphatides. "Linker" bases or
combinations of linker bases would be represented on different
oligonucleotides sequences. New molecules can then be built from
10   these shorter sequences using crossover amplification strategies
or ligase strategies described above.

In another embodiment one or more of said agent molecules in step
(c) are different from the agent molecules utilized in the
15   morphatides of the prior round of screening for identification
of morphatides performing the preselected or desired function.

In another embodiment of the above-described methods for
identifying a different morphatide further comprising: (a)
20   separating the scaffolding components from the agent molecules;
(b) performing a suitable number of cycles of error prone PCR on
the scaffolding components, resulting in one or more base changes
in some percentage of the scaffolding components; (c)
reconnecting the scaffold component to the agent component; and
25   (d) repeating steps (a) through (d) of the above-described
methods, thereby identifying a different morphatide. In an
embodiment the morphatide comprises a linker component, wherein
in step (a) one part of a linker remains attached to the scaffold
component and another part of the linker remains attached to the
30   agent molecule. In another embodiment in step (c) both parts of
the linker are connected, thereby reconnecting the scaffold
component to the agent component. In a further embodiment the
connection between the agent molecule and the scaffolding
component is by a plurality of the linker component, i.e., pieces
35   of the linker component. In an embodiment the scaffolding
components are characterized by cloning and nucleotide sequencing

40

before reattachment of the agent molecule in step (c).

A further embodiment of the above-described methods comprises (a) creating a mimic of the identified morphatide; and (b) using the
5   mimic for a desired application.

In a still further embodiment the above-described methods further comprise: (a) separating scaffolding components with attached linker components or parts of the linker components from the
10   agent molecules of the previously identified Morphatides; (b) combining the scaffolding components with attached linker components or parts thereof with scaffolding components comprising a same nucleic acid sequence as the scaffolding components, said nucleic acid sequence not being attached to or
15   associated with a linker components or parts thereof, thereby resulting in nucleic acid sequences without the one or more linker sites; (c) using sexual PCR to fragment and reassemble the nucleic acid sequences, resulting in elimination of linker component sites which do not contribute to the binding of the
20   morphatide, thereby generating new scaffolding components similar but not identical to the scaffolding components of step (a); (d) reattaching or association agent molecules to the new scaffolding components of step (c), thereby generating another set of Morphatides.
25

Yet another aspect of the present invention provides a method of identifying a presence of a substance in a sample from a subject, comprising: (a) obtaining a sample; (b) contacting the sample with one or more types of morphatide identified by either of the
30   methods for identifying one or more complexes so as to form a complex between the morphatide and the substance present in the sample; (c) detecting the complex formed in step (b), thereby identifying the presence of the substance in the sample from the subject.   In an embodiment step (c) is performed by PCR
35   amplification, ethidium bromide staining or labeling selected

41

from the group consisting of radioactive isotope, enzyme, dye, biotin, a fluorescent label, a chemiluminescent label and a ligand. In a preferred embodiment the detection of the complex formed in step (c) comprises identification of an occurrence
5   selected from the group consisting of binding to or associating with a target molecule; having an affinity for and binding to a specific ligand; performance of a biological activity, wherein said biological activity is selected from the group consisting of antimicrobial activity, antitumor activity, enzyme inhibiting
10   activity, enzyme enhancing activity, receptor binding activity, growth promotion activity, antibody binding activity; formation of a biofilm; enzymatic activity, immune modulating activity, cell signaling activity, polymerizing activity, and encapsulating activity.
15

This invention further provides a method of diagnosing a subject wherein detection of a complex in step (c) of a method of identifying a presence of a substance in a sample is indicative of a disease. In an embodiment the sample is a body fluid or a
20   tissue specimen. In another embodiment the body fluid is selected from the group consisting of blood, serum, plasma, urine, saliva, nasal mucosal discharge, vaginal mucosal discharge, anal mucosal discharge, peritoneal fluid, cerebro-spinal fluid, and lymphatic fluid. In a further embodiment the
25   substance whose presence is identified is selected from the group consisting of hormones, enzymes, proteins, cancer/tumor cells, pathogens, and drugs. In a still further embodiment the subject is bacterial cells, a plant, a microbe, an insect, a fish, or a mammal. In a preferred further embodiment the mammal
30   is a human.

Still another aspect of the present invention provides a morphatide capable of effectively binding to, crosslinking with, or reacting with multiple types of molecules. Another aspect of
35   the present invention provides a morphatide capable of effectively binding to, crosslinking with, or reacting with one

type of molecule.

In a preferred embodiment of the morphatide the binding to, crosslinking with, or reacting with the molecules is selected

5 from the group consisting of binding to or associating with a target molecule; having an affinity for and binding to a specific ligand; performance of a biological activity, wherein said biological activity is selected from the group consisting of antimicrobial activity, antitumor activity, enzyme inhibiting

10 activity, enzyme enhancing activity, receptor binding activity, growth promotion activity, antibody binding activity; formation of a biofilm; enzymatic activity, immune modulating activity, cell signaling activity, polymerizing activity, and encapsulating activity.

15

This invention provides a composition comprising the preferred embodiment of the morphatide effective to treat a subject and a pharmaceutically acceptable carrier.

20 This invention also provides a method of administering the above-described compositions, wherein the administration is intravenous, intraperitoneal, intra-thecal, intralymphatical, intramuscular, intralesional, parenteral, epidural, subcutaneous; by infusion, liposome-mediated delivery, aerosol delivery;

25 topical, oral, nasal, anal, ocular or otic delivery. In a preferred embodiment the morphatide is conjugated to a therapeutic agent. In an embodiment the therapeutic agent is a radioisotope, toxin, toxoid, or chemotherapeutic agent.

30 This invention further provides a composition comprising the above-described conjugated morphatide and a pharmaceutically acceptable carrier, wherein the morphatide is selected from either a morphatide which is capable of being degraded or a morphatide which is incapable of being degraded after

35 administration to a subject.

43

The present invention also provides a pharmaceutical composition comprising a effective amount of the morphatides described above and a pharmaceutically acceptable carrier. In the subject invention an "effective amount" is any amount of an morphatide

5   which, when administered to a subject suffering from a disease or abnormality against which the morphatides are effective, causes reduction, remission, or regression of the disease or abnormality. In the practice of this invention the "pharmaceutically acceptable carrier" is any physiological

10 carrier known to those of ordinary skill in the art useful in formulating pharmaceutical compositions.

In one preferred embodiment the pharmaceutical carrier may be a liquid and the pharmaceutical composition would be in the form

15 of a solution. In another equally preferred embodiment, the pharmaceutically acceptable carrier is a solid and the composition is in the form of a powder or tablet. In a further embodiment, the pharmaceutical carrier is a gel and the composition is in the form of a suppository or cream. In a

20 further embodiment the compound may be formulated as a part of a pharmaceutically acceptable transdermal patch.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending

25 agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material.
In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the

30 necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin,

35 cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an

5   organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators,

10 stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols,

15 e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration.

20 The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellent.

Liquid pharmaceutical compositions which are sterile solutions

25 or suspensions can be utilized by for example, intramuscular, intrathecal, epidural, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. The compounds may be prepared as a sterile solid composition which may be dissolved or suspended at the time of administration using

30 sterile water, saline, or other appropriate sterile injectable medium. Carriers are intended to include necessary and inert binders, suspending agents, lubricants, flavorants, sweeteners, preservatives, dyes, and coatings. The compositions may be further comprised of stabilizers, which as used herein, is a

35 substance which increases the half-life of the morphatide in the blood stream. Polyethylene glycol may be used as a stabilizer. A stabilizer would comprise the composition, for example when

45

the morphatide of the composition is to be degraded after administration so as to deliver a bound therapeutic agent which is to be time released.

5   The morphatides can be administered orally in the form of a sterile solution or suspension containing other solutes or suspending agents, for example, enough saline or glucose to make the solution isotonic, bile salts, acacia, gelatin, sorbitan monoleate, polysorbate 80 (oleate esters of sorbitol and its
10 anhydrides copolymerized with ethylene oxide) and the like.

The morphatides can also be administered orally either in liquid or solid composition form. Compositions suitable for oral administration include solid forms, such as pills, capsules,
15 granules, tablets, and powders, and liquid forms, such as solutions, syrups, elixirs, and suspensions. Forms useful for parenteral administration include sterile solutions, emulsions, and suspensions.

20 Optimal dosages to be administered may be determined by those skilled in the art, and will vary with the particular morphatide in use, the strength of the preparation, the mode of administration, and the advancement of the disease condition or abnormality. Additional factors depending on the particular
25 subject being treated will result in a need to adjust dosages, including subject age, weight, gender, diet, and time of administration.

The present invention provides a morphatide labelled with a
30 detectable marker. In an embodiment the detectable marker is selected from the group consisting of a radioactive isotope, enzyme, dye, biotin, a fluorescent label, a chemiluminescent label and a ligand.

35 In an embodiment of the above-described compositions wherein the morphatide is incapable of being degraded the composition further comprises a stabilizer molecule for increasing the half-life of

46

the morphatide in the blood stream. In an embodiment the
stabilizer is polyethyleneglycol.

5   This invention provides a method of treating a subject with any
     of the above-described compositions of morphatides and
     morphatides conjugated to therapeutic agents.

     In another aspect, this invention provides a method of drug
     delivery to a target in the body of a subject comprising
10   administration to a subject any of the above-described
     compositions of morphatides and morphatides conjugated to
     therapeutic agents, thereby delivering the drug to the target.

15   In an embodiment of the above-described compositions wherein a
     morphatide is capable of being degraded, the degradation is
     performed by either of a nuclease or protease.

     This invention further provides a method of drug delivery to a
20   target in the body of a subject comprising administration to a
     subject of the above-described compositions of morphatides and
     morphatides conjugated to therapeutic agents, wherein the
     morphatide is incapable of being degraded or is slowly degraded
     after administration to the subject, thereby delivering the
25   morphatide-bound drug to the target. In an embodiment of the
     method of drug delivery, wherein the morphatide-bound drug is
     administered, the administration may be intravenous,
     intraperitoneal, intrathecal, intralympha-tical, intramuscular,
     intralesional, parenteral, epidu-ral, subcutaneous; by infusion,
30   liposome-mediated delivery, aerosol delivery; topical, oral,
     nasal, anal, ocular or otic delivery.

     In a further embodiment of the above described methods of
     identifying morphatides, the morphatide is capable of binding to
35   any component of an antibody molecule, said antibody having a
     constant and variable region.

47

It is contemplated that products of the present invention are useful in dideoxy-sequencing, cycle-sequencing, restriction enzyme based and other similar sequencing strategies, the methods of which are well known in the art.

In standard dideoxy sequencing reactions, template nucleic acid molecules are incubated in four separate reactions with primers, nucleotides (all four), and dideoxy (chain terminator) nucleotides (different dideoxy nucleotide for each reaction). After primer hybridization, polymerase is added to the mixture and extension proceeds. The incorporation of a dideoxy nucleotide terminates the extension reaction. The presence in a reaction of both regular nucleotide and the terminator version of a nucleotide ensures that the termination event will not occur every time the nucleotide is required to be incorporated. Thus, this random incorporation of dideoxy nucleotides yields fragments, which can be distinguished based on size when denatured and separated by electrophoresis. The procedure is described in detail in Sambrook, Fritsch, Maniatis, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989.

Fragments generated by the above method are labeled for detection by a variety of methods. Radioactive, or fluorescent or infrared dye labels are employed. Primers are labeled, or chain terminating molecules (for example, dideoxy nucleotides) are labeled. One problem encountered with labeling chain terminator molecules is that polymerases do not generally incorporate each terminator molecule with equal efficiency. Nucleotides or nucleosides associated with linker molecules in accordance with the methods of the present invention can be utilized in sequencing reactions to overcome this problem. Polymerases can incorporate nucleotides associated with linker moieties with equal efficiency. Detectable molecules, such as agent molecules linked to fluorescent dyes, or fluorescent molecules themselves, can be linked to the nucleotide (or nucleoside)/linker molecules

after the sequencing reaction to allow detection of the nucleic acid fragment. Nucleotide/linker moieties may be incorporated into the sequencing primers, or may be incorporated during the sequencing reaction, internally, or in the form of a chain

5   terminator (for example, a dideoxy molecule associated with a linker). It is also contemplated that nucleotide/linker/ detectable moiety molecules could be incorporated into an extending chain with acceptable efficiencies, thus have utility in sequencing reactions.

10

It will be appreciated to one skilled in the art that the methods of the present invention have several advantages over existing technologies. Among these advantages are the following: agent molecules and scaffolding molecules can be cycled separately or

15   together; a variety of agent molecules and scaffolding molecules can be utilized; in many screening processes, reaction conditions (such as temperature or salt conditions) can be readily modified to enrich, screen, or select for particular association events; linker molecules can be utilized to generate

20   novel secondary and tertiary structures; this is a cell free system, helping to avoid potential background contamination or false results.

In particular, if scaffolding molecules used are nucleic acids,

25   the following advantages are afforded: single stranded, double stranded, triple stranded, or branched nucleic acids can be utilized; analogue nucleic acid molecules can be utilized, further increasing the potential structural diversity of the scaffolding molecule; linker molecules or components of linker

30   molecules can be attached to multiple bases in the nucleic acid molecules; mixtures of bases can be utilized; sequencing can be employed to decipher desirable or resulting scaffolding molecules; mutations can be focused on subdomains within the nucleic acid molecules to modify the scaffolding molecule as

35   desired, if so desired; modified polymerases can be employed for

49

wider utility in the generation of nucleic acid scaffolding molecules, and in the modification process, if this process requires the use of polymerases; and non-degradable nucleic acids can be utilized in this method.

5

These and other aspects of the present invention will be apparent to those skilled in the art.

This invention will be better understood from the Experimental
10 Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

15   **EXAMPLES**

Example 1
Generation and Amplification of Linker Conjugated Scaffolding Molecules
20
1) Three pools of nucleic acid scaffolding molecules are generated using sequence randomized template nucleic acids generated (using solid state phosphoamidite chemistry followed by PCR amplification) with phenylboronic acid linker reagent
25 conjugated dUTP molecules and dUTP, dATP, dCTP, and dGTP. Conjugated dUTP molecules are generated utilizing standard chemistry and phenylboronic acid linker complexing reagents. Different ratios of conjugated dUTP molecules to unconjugated dUTP molecules are present in each pool. Pool #1 contains
30 conjugated:unconjugated dUTP present in a 1:10 ratio; pool #2 contains conjugated:unconjugated dUTP present in a 1:1 ratio; and pool #3 contains conjugated:unconjugated dUTP present in a 10:1 ratio. Error prone PCR is performed on each pool.

50

Generation of Morphatides

2) Five different conjugated amino acids (leucine, aspartic acid, glutamine, phenylalanine and tyrosine) (generated utilizing
5   standard chemistry and phenylboronic acid complexing reagents) are combined to generate a mixture of conjugated agent molecules.

Condensation reactions are performed utilizing the resulting mixture and each of the three pools, generating complexes of scaffolding molecules/linker molecules/agent molecules.

10   Selection and Evolution of Morphatides

3) Selection of thrombin binding Morphatides is performed on each pool as described infra in Example 4.

15   4) Scaffolding molecules are separated from agent molecules by reversing the linker via a small shift in pH and temperature, and cloned and sequenced as in Example 6.

5) Scaffolding molecules with the same or similar sequences as
20   those obtained in Step 4 are generated via standard solid phase phosphoamidite chemistry, as in step 1, without the use of conjugated nucleotides.

6) Scaffolding molecules of selected Morphatides are then
25   subjected to sexual PCR according to the following protocol to generate modified versions of the selected molecules:

   a) Double stranded DNA from each pool and double stranded DNA from Step 6 are amplified, and free primers are removed from the samples;

30   b) About 5 μg of the DNA from each sample is digested with 0.15 units of DNAse I (Sigma, St. Louis, MO) in 100 μl of [50mM Tris-HCl ph 7.4, 1 mM MgCl$_2$], for 10-20 minutes at room temperature. The digested DNA is run on a 2% low melting point agarose gel. Fragments of the desired size

51

range are purified from the 2% low melting point agarose gel by electrophoresis onto DE81 ion exchange paper (Whatman, Hillsborougy, Oregon). The DNA fragments are then eluted from the paper with 1M NaCl and ethanol

5   precipitated.

c) Purified fragments are resuspended at a concentration of 10-30 ng/μl in PCR Mix (0.2mM each dNTP, 22 mM $MgCl_2$, 50 mM KCl, 10mM Tris-HCl ph 9.0, 0.1 % Triton X-100, 0.3 μl Taq DNA polymerase, 50 μl total volume). A reassembly program

10   of 94°C for 60 seconds, 30-45 cycles of [94°C for 30 seconds, 50-55° C for 30 seconds, 72°C for 30 seconds] and 5 minutes at 72°C is used in a thermocycler. Reaction can be followed by taking samples after 25, 30, 35, 40 and 45 cycles of reassembly.

15

Morphatide complexes are regenerated by complexing to agent molecules and new Morphatides are rescreened for binding to thrombin. Process is performed as many times as needed to narrow pool to most desirable Morphatides.

20

Example 2
Generation and Amplification of Scaffolding Molecules

Generation of Scaffolding Molecules
25   Four libraries of scaffolding molecules containing a random region (variable cores) and constant flanking regions are constructed. In each case, three of the four bases are incorporated with similar frequencies and one base is represented in a much reduced amount (e.g., $1/10^{th}$ of the other three bases,

30   although this ratio depends upon several factors, including the length of the variable core), hereinafter the restricted or rare base. The restricted base later provides the template for the incorporation of the linker base or nucleotide associated with linker molecules (a linker base can be a base analog as well).

52

This approach permits the random modification of the variable cores in a manner that permits the position of the linker bases to be determined. This strategy is termed the 4x(3+1).

5   In a 4x(3+1) strategy, four pools of ssDNA oligomers with 72 nucleotides are prepared using solid state phosphoamidite chemistry. Eighteen pool specific base positions at the 5' and 3' end are kept constant for primer recognition during PCR. The central 36 positions are randomized by incorporating 3 bases at
10   31.3% each and one base, containing the linker, at 5%. After deprotection from the solid support, the pools are purified and recovered by precipitation using standard oligonucleotide purification protocols.

15   Therefore, scaffolding molecule sequences consist of three regions: 1) a fixed 5' sequence of 18 nucleotides, 2) a randomized middle part of 36 nucleotides, and 3) a fixed 3' sequence of 18 nucleotides. The two fixed sequences serve as PCR primer anchor sites. The variable core is synthesized as
20   randomized sequences in four groups or pools. Variable cores in the first pool are synthesized with adenine reduced to 5% of the other bases, in the second pool with similarly reduced G, in the third pool with reduced % C, and in the fourth pool with reduced % T. These are termed the 3+1 reactions indicating that three of
25   the bases are represented in equal amounts while each of the other nucleotides are present at the reduced level. In this manner, four pools of oligonucleotides are created. The rare base is the one that contains the linker molecule that can be connected or associated to agent molecules after each round of
30   amplification-selection. It is the infrequent appearance of this base that reveals the potential sites of association or connection.

The two primer sequence anchors are designed as to not contain
35   the nucleotide that is present in low concentrations in the variable core region. As a consequence, each pool will be anchored by pool specific primer pairs. Linker bases will thus

only occur in the random core.   This allows the determination of
the positions of linker bases by DNA sequencing.

Amplification of Random Pools by PCR

5

The four pools are subjected individually to PCR amplification
using two primers homologous to the anchored sequences.   A
selection protocol for single stranded DNA (ssDNA) is used. (Bock
et al. 1992).   These protocols are known in the art.   One primer
10   (the reverse primer, corresponding to the complement of the 3'
end of the oligomer pools) is biotinylated to allow for later
isolation of a single strand. For the rare base [that is present
at low levels] in each of the four pools, a nucleotide is used
that has the linker group attached. Hence, the rare nucleotides
15   in the 36 nucleotide variable cores in the four pools of
oligonucleotides have linker moieties associated or connected to
them.   The   scaffolding   molecules   are   then   applied   to   a
streptavidin-agarose column (equilibrated to 0.1 M Tris-HCl, pH
7.5, 0.1 M NaCl) (Griffin et al., 1993; Bock et al., 1992), and
20   ssDNA (corresponding to original sequence library)   eluted with
0.15 N NaOH. The flow through fraction (un-biotinylated strand)
is collected, neutralized with acetic acid, concentrated and
precipitated with ethanol.

25   **Example 3**
Generation of Morphatides

The rare DNA bases in the four random pools containing linkers
are now individually reacted with the agent molecule threonine
30   via a coupling reaction to generate the morphatides.

Selection of agent molecules to be used can depend on several
factors.   For example, aliphatic pentynyl groups can provide a
site for hydrophobic interaction with a hydrophobic cluster of
35   surface residues on thrombin. Knowledge of essential structures
on the target molecule, such as catalytic site may be helpful in

considering agent molecules. Of general use are charged groups such as several amino acids (Asp, Glu, and Arg) and hydrophobic moieties such as hydrophobic amino acids (Trp, Tyr, and Phe). However, many similar chemical groups are worthwhile considering.

5   Small peptides are possible too.

Example 4

Selection of Morphatides with Affinity to Thrombin

10   After PCR amplification the four pools of morphatides are combined. Selection cycles are performed on concanavalin A column immobilized human thrombin (Bock et al. 1992). The DNA from Example 2 is precipitated and dissolved in selection buffer: (20 mM tris-acetate, pH 7.4, 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$). The

15   DNA is first applied to a concanavalin A-agarose column to remove those molecules that recognize concanavalin A or agarose structures. The flow through is then applied to a column containing thrombin bound to the concanavalin A - agarose support. The column is washed several times with selection

20   buffer and the binding morphatides (selected morphatides) are eluted with 0.1 M α-methylmannoside, in the selection buffer (Griffin et al., 1993; Bock et al., 1992).

Example 5

25   Evolution of Selected Morphatides

Selected morphatides are subjected to phenol extraction to remove the thrombin. Scaffolding components are separated from the threonine molecules. The resulting scaffolding molecules are precipitated with 20 µg glycogen and three volumes of ethanol.

30   After resuspending the DNA, the oligomer solution is split into four pools, and amplified under error prone conditions. High fidelity amplification is utilized for the final amplification step, i.e. first sloppy PCR and then high fidelity PCR. For each pool the corresponding restricted nucleotide and the appropriate

35   PCR primer sets are used. After PCR amplification the fragments

are again reacted with the agent molecules. (Griffin; Bock) A
total of six selection cycles over conA-agarose are performed.
On average five cycles perform well.

5   **Example 6**

Cloning and Sequencing of the Scaffolding Components of High
Affinity Morphatides

Characterization of the scaffolding components of the high
10   affinity thrombin binding Morphatides after the final selection
step is performed by cloning and sequencing of the nucleic acids.
Single-stranded DNA (of the scaffolding components) is converted
to dsDNA by PCR and cloned into an M13mp18 based TA modified
cloning vector commercially available (Invitrogen Corp.). This
15   vector also serves as a convenient repository for the generation
of oligomers by PCR and generation of template for automated
rapid DNA sequencing using robust M13 technology. This cloning
and sequencing provides the DNA sequences of the selected
scaffolding molecules, and, as only the rare nucleotide in each
20   pool has the linker attached, also reveals the information about
the position of the agent molecule. The sequencing results from
the four random pools are analyzed using DNA alignment and
phylogenetic software (widely available and utilized) to
establish consensus sequences of the dominant binders.
25

Example 7

Characterization of Thrombin Specific Morphatides

Determination of Binding Characteristics
30

Binding constants to thrombin are determined. Because a
considerable number of binding and competition experiments are
performed, a rapid assay is employed. Thrombin is immobilized
on microtiter plates (Tsiang et al. 1995b) and radioactively
35   labeled Morphatides are added in series of different
concentrations. After washing steps, the determination of binding

constants is performed using rapid computerized phosphoimager technology. Washing steps are well known to one of ordinary skill in the art. Microtiter plates can be prepared in series and stored at 4°C for several weeks and the experiments performed

5   using 96-well automated pipetting and washing steps. Crucial experiments to investigate cooperative binding effects, competitive binding and number of binding sites will be performed by equilibrium dialysis methods.

10   **Example 8**

Preparation of A Synthetic Oligonucleotide Reactive Phenylboronic Acid Complexing Reagent of General Formula XIX and Application Thereof (WO 95/20591, Phenylboronic Acid Complexes, August 3, 1995)

15

In the initial step of the synthesis 2-[2-(2-chloroethoxy]ethanol is condensed with *N*-hydroxy-phthalimide by refluxing in dimethylformamide containing one equivalent of triethylamine for 2 days. The product is precipitated by pouring into water,

20   collected by filtration, washed with water, dried in a vacuum dessicator, and used without further purification.

In the second step of the synthesis, the crude product obtained above is refluxed briefly in a mixture of acetic acid and

25   concentrated hydrochloric acid. After cooling, the precipitated phthalic acid is filtered from solution and the filtrate concentrated and then coevaporated repeatedly from small volumes of water to remove traces of acids. Finally, the aminooxy hydrochloride product is neutralized with NaHCO$_3$, extracted in

30   ethyl acetate, dried over anhydrous MgSO$_4$, and concentrated in vacuo.

Synthetic oligonucleotides may be conjugated with a 2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite phenylboronic acid complexing

35   reagents, during the final step of an automated solid-phase oligonucleotide synthesis, to afford synthetic oligonucleotides

57

having 5'- pendant phenylboronic acid complexing moieties.

Example 9

5   The following is a further example of the general methodology for
     the generation and screening of Morphatides.

General Outline of the Steps Performed:

     1.   Synthesis of degenerate single stranded DNA oligonucleotide
10   2.   Chemical synthesis of DNA base derivative which serves as
          the "linker base"
     3.   Amplification of degenerate DNA oligonucleotide from step
          1 by the polymerase chain reaction (PCR) including
          incorporation of the linker base during amplification
15   4.   Generation of single stranded DNA from the PCR product using
          immobilized avidin carriers
     5.   Chemical coupling of agent molecules to the single stranded
          DNA from step 4 to generate pools of Morphatides consisting
          of oligonucleotide scaffolding components, linker
20   components and agent molecules attached at the positions of
          the "linker base"
     6.   Immobilization of target molecules on solid support
     7.   Screening: selection of Morphatides with affinity to the
          immobilized target by mixing of the pool from step 5 with
25   the immobilized target and washing away the unbound
          material
     8.   Evolution of scaffolding components of selected Morphatides
          (bound to target) by shuffling or sloppy PCR
     9.   Repeat steps 3 through 8 to select Morphatides with high
30   affinity to the selected target molecules

Experimental Details:

     1.   Synthesis of degenerate single stranded DNA oligonucleotides
35   (template to generate the scaffolds): Synthesis and purification
     of a degenerate oligonucleotide pool of the type A-Z-B is

performed by standard methods (phosphoamidate chemistry, trityl off/on cartridge purification, HPLC, capillary electrophoresis). A denotes the following constant 18-mer 5' flanking region of the sequence: 5'AAA CCA GCA AAA ACA AAA3'; Z denotes a variable

5   34-mer core, called the "Morphacore" sequence, with A, C, G, and T occurring in variable but typically equal ratios at every position; and B denotes the following constant 18-mer 3' flanking region of the sequence: 5'AGA AAG AAA GAG CAA ACA3'

10   2.   Chemical synthesis of DNA base derivatives which serve as the "linker base": Linker bases are synthesized using standard chemistry techniques. Phenylboronic acid derivatives of DNA bases (phenylboronic acid dUTP) are commercially available from Prolinx, Bothell WA; SPDP (N-succinimidyl 3-

15   [pyridyldithio]propionate) derivatives of DNA (SPDP dUPT) bases can be synthesized commercially by Dalton Chemicals, Toronto ON, Canada.

3.   Amplification of degenerate synthetic DNA oligonucleotide
20   from step 1 by polymerase chain reaction (PCR) including incorporation of the linker base during the amplification reaction:
PCR is performed under the following conditions:
250 μM of each dATP, dGTP, dCTP
25   250 μM phenylboronic acid dUTP
0.5 μM forward primer 5'AAA CCA GCA AAA ACA AAA3'
0.5 μM reverse primer 5' biotin-TGT TTG CTC TTT CTT TCT3'.
5 mM tris(hydroxymethyl) aminomethane/HCl pH 9.0
60 mM potassium chloride
30   10mM magnesium chloride
2 ng/μl 70mer degenerate oligonucleotide from step 1

To generate DNA for a typical selection round, 5 μg of template should be used (~10^{14} molecules)
35   10 units of Taq polymerase per 100 μl assay volume

Amplification of DNA is performed in a thermocycler (PerkinElmer

2400) with the following program: 94°C for 1 min followed by 30 cycles of 94°C for 10 seconds, 55°C for 20 seconds and 72°C for 20 seconds, followed again by 72°C for 1 min and a 4°C hold.

PCR products are analyzed on 2.5% PCR agarose gels (Invitrogen

5   Corporation) and 10% polyacrylamide 7M urea gels (Novex, San Diego Ca.) and purified and concentrated by standard 2 volume 100% ethanol precipitation followed a 70% ethanol wash. After drying and rehydration the DNA is redissolved in 10mM tris(hydroxymethyl) aminomethane/HCI, 1μM ethylenediamine

10   tetraaceticacid pH 8.0.

4.   Generation of single stranded DNA from the PCR produce using immobilized avidin carriers: Single stranded Morphocore molecules are separated from the complement strands (reverse

15   strand) by absorbing the biotinylated PCR product to Avidin agarose beads.   The non biotinylated Morphacore strand is then melted off, and collected. Typically, 100μg of PCR product (from step 3) in 10 mM Tris(hydroxymethyl) aminomethane/HCI pH 8.0 (Tris pH 8.0) is batch bound to 500μl of avidin coupled resin in

20   a 1 ml volume for 15 min at room temperature.   This slurry is then transferred to a microcentrifuge spin column 7, and spun at 500xg for 1 min. The column is then washed with 2ml of 10mM Tris pH 8.0 by sequential resuspension of the resin in 1 ml, followed by centrifugation. The DNA single strands are eluted in a final

25   volume of 1.8ml with 10-30mM NaOH by sequential resuspension of the resin in 0.9 ml, followed by centrifugation. The 0.9 ml elutions are neutralized by spinning into a collection tube containing 0.1ml 10mM Tris (pH unadjusted) and 10-30mM hydrochloric acid. Each fraction is analyzed by electrophoresis

30   on a 10% polyacrylamide gel containing 7M urea (Novex), and visualized by ethidium bromide staining.

5.   Chemical coupling of agent molecules to the single stranded DNA from step 4 to generate pools of Morphatides consisting of

35   oligonucleotide scaffolding components, linker components and agent molecules attached at the positions of the "linker base":

60

Single stranded DNA with phenylboronic acid covalently linked to DNA bases generated in step 4 is used as a substrate for the coupling of agent molecules. Agent molecules are groups (for example amino acids) which are covalently linked to salicyl
5   hydroxamic acid functional groups. The salicyl hydroxamic acid will form a reversible complex with the phenylboronic acid moieties of the linker bases in the DNA. Coupling is performed in 0.1 M sodium bicarbonate pH 9.7 at 37°C for 24 hours. Concentration of the reactants is 1 mM of the salicyl hyroxamic
10   acid derivative of the agent molecule and 100 μg per ml of single stranded DNA from step 4. After the incubation period, the coupling reaction is purified by commercially available SNAP purification kits (Invitrogen Corporation, Carlsbad, CA) which separate the reaction product (the Morphatides) from the
15   unreacted salicyl hydroxamic acid monomeric binder molecules. Morphatides are eluted from the spin columns, ethanol precipitated and resuspended in the selection buffer used in step 7 below.

20   6.   Generation of Immobilized targets.
     Polypeptide target molecules are covalently attached to 3.76 μm carboxyl magnetic particles 1 via a two step coupling procedure2 using the carbodiimide EDAC [1-ethyl-3-(3 diethylaminopropyl) carbodiimide hydrochloride]3 and sulfoNHS (N-
25   Hydroxysulfosuccinimide) 4method. For a typical selection 1x10^9 beads are reacted in 10ml 50mM MES (2-[N-Morpholino]ethanesulfonic acid), 500mM NaCl, pH6.0 containing 2mM EDAC, and 5mM sulfoNHS for 15 min at room temperature to allow formation of the sulfoNHS activated ester intermediate. The
30   beads are then washed 3 times with 10 ml coupling buffer (50 mM potassium phosphate pH 7.5), resuspended in 10 ml coupling buffer containing 10μM target molecules, and allowed to react for 2 hours at room temperature. The beads are then washed 3 times with 10 ml coupling buffer and reacted in coupling buffer
35   containing 200μM ethanolamine for two hours at room temperature to block any remaining reactive groups. The reacted and blocked

beads are then washed 6 times in 10 ml coupling buffer and stored at 4°C at a concentration of 1x10^8 beads/ml in coupling buffer.
Control beads are prepared in the same manner as above, substituting ethanolamine for the target molecule.

5

To estimate the number of target molecules coupled to the beads, a Bichinconic acid protein assay 5 is performed on 1x10^7 beads. The supernatant from the bead/assay mixture is read at 570 nm and compared to a standard curve to calculate the quantity of
10 protein coupled to the beads. Given the molecular weight of the coupled polypeptide the number of molecules per bead is calculated. Typical coupling efficiencies with this protocol are 10^6 molecules coupled per magnetic latex bead.

15 7.   Selection of Morphatides with affinity to the immobilized target by mixing of the pool from step 5 with the immobilized target and washing away the unbound material:
The coupled Morphatide pool from step 5 is used for selection of target molecules coupled to magnetic beads as described in step
20 6. The selection buffer used is 20 mM Tris/acetate pH 7.2, 140 mM NaCl. The number of latex beads coupled with target molecules used in the first round of selection is calculated to provide one target molecule per Morphatide sequence represented in the pool.
Thus at a coupling efficiency of 10^6 molecules of target per
25 magnetic latex bead and a library diversity of 10^{14} molecules, 10^8 beads are used in the first round of selection.
As a first step, Morphatides which bind unspecifically to the carrier are removed from the pool by adding 10^8 control coupled beads to the pool. The mixture is incubated at room temperature
30 for 4 hours with gentle shaking, the beads are collected at the side of the tube with a suitable magnet and the supernatant containing unbound Morphatides is removed. This Morphatide pool is added to the coupled latex beads (0.5 ml) and incubated at room temperature for 16 hours with gentle shaking (platform
35 shaker). After this binding step the latex beads are washed five times with 2 ml of selection buffer, again using a magnet to

collect the beads at the side of the tube. The supernatants from the washing steps are combined in a tube and latex beads are collected at the side of the tube, washed, and combined with the bulk of the beads to avoid losses during the washing steps.

5   Morphatide molecules bound to targets are released by resuspending the beads in 2% sodium dodecylsulfate and heating to 90°C for 10 min. After collecting the beads the supernatant is extracted with phenol/chloroform and ethanol precipitated. The pellet after ethanol precipitation is resuspended in 20 μl

10   of 10 mM Tris/HCI pH 8.5 and used as template in a sloppy amplification reaction or shuffling reaction as per step 8 below.

8.   Evolution of scaffolding components of Morphatides bound to target by shuffling or sloppy PCR Morphatides released from the

15   target in step 7 are separated into scaffolding components and agent molecules, and the scaffolding components are subjected to amplification "sloppy PCR" reaction for evolution. Scaffolding components can also be "shuffled" by suspending the fragments in a PCR mix (0.2 mM each dNTP, 2.2 mM MgCl$_2$, 50mM KC1, 10mM Tris-

20   HCI pH 9.0, 0.1% Triton X-100), at a concentration of 10-30ng/μl and amplifying under the following conditions: 94°C for 60 seconds, 40 acycles of 94°C for 30 seconds, 50-55°C for 30 seconds, 72°C for 30 seconds followed by a 5 minute incubation at 72°C in any standard thermocycler.

25

9.   Repeat steps 3 through 8 to select Morphatides with high affinity to the selected target molecules. In subsequent rounds of selection the same methods are employed as described above. However, in order to introduce moderate selective pressure into

30   the rounds of selection, the number of target molecules coupled to magnetic latex beads is reduced by a factor of two for each selection rounds, thus in a typical series of experiments with 8 round of selection the number of targets is reduced by a factor of 128 (the complete process to select Morphatides using this

35   method is termed exponential morphatide selection and evolution, EMSE). To follow the progress of the selection, Morphatides

released from the target after round two are routinely cloned and sequenced. Typically double stranded linear DNA after the two stage PCR is cloned into a topoisomerase clogging vector (Topo 1, Invitrogen) with the capacity to allow direct selection for inserts. 30 independent clones are isolated and sequenced per selection round.

1. Spherotech Inc. Libertyville, Illinois cat no. CM-30-50
2. Protocol adapted from GT Hanson, Bioconjugate Techniques, Academic press inc, 1996, pp 176.
3. Sigma chemical company, St. Louis, Missouri, cat no. E 1769
4. Pierce Chemical Company, Rockford, Illinois, cat no. 24510
5. Pierce Chemical Company, Rockford, Illinois, cat no. 23223
6. Vector Laboratories, Burlingame, California, cat no. A-2010
7. Costar Corp., Cambridge, Massachusetts cat no. 8170

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PCT/US94/10562 filed September 19, 1994.

International Patent Application WO 96/09316, entitled "Parallel Selex", March 28, 1996.

International Patent Application WO 95/20591, entitled "Phenylboronic Acid Complexes", August 3, 1995.

What is claimed is:

1. A method for identifying one or more complexes from a library of complexes, wherein said complex or complexes are selected for their ability to perform a preselected or desired function on a target molecule or by having a pre-selected structure, each complex being designated a morphatide, said method comprising:

(a) preparing a library of morphatides, comprised of:
(i) a scaffolding component selected from the group consisting of nucleic acid, nucleic acid like molecule or nucleic acid analog having one or more regions of randomized sequence;
(ii) one or more linker components; and
(iii) one or more agent molecules or type of agent molecules, linked to the scaffolding component by one or more type of linker components; and

(b) screening the library of morphatides prepared in step (a) by contacting, binding, or associating the morphatides with one or more suitable target molecules upon which a morphatide performs a preselected or desired function or to which a morphatide binds or associates through a pre-selected structure of said morphatide under conditions permitting said morphatide to perform said preselected or desired function on said target molecules or permitting said morphatide to bind or associate with said target molecules through the preselected structure;

(c) separating the morphatides performing the preselected or desired function or binding or associating through the preselected structure, from the library of morphatides and target molecules; thereby identifying one or more complexes from a library of complexes, wherein said complex or complexes are selected for

their ability to perform a preselected or desired
function on a target molecule or by having a pre-
selected structure.

5   2.   A method for identifying one or more complexes from a
          library of complexes, wherein said complex or complexes are
          selected for their ability to perform a preselected or
          desired function on a target molecule or by having a pre-
          selected structure, each complex being designated a

10   morphatide, said method comprising:

          (a) preparing a library of morphatides, comprised of:
                  (i) a scaffolding component selected from the group
                  consisting of nucleic acid, nucleic acid like

15   molecule or nucleic acid analog having one or more
                  regions of randomized sequence; and (ii) one or more
                  agent   molecules   or   type   of   agent   molecules,
                  associated, bound, or bonded to the scaffolding
                  component;

20   (b)   screening the library of morphatides prepared in step
                  (a) by contacting, binding, or associating the
                  morphatides with one or more suitable target molecules
                  upon which a morphatide performs a preselected or
                  desired function or to which a morphatide binds or

25   associates through a pre-selected structure of said
                  morphatide under conditions permitting said morphatide
                  to perform said preselected or desired function on
                  said target molecules or permitting said morphatide to
                  bind or associate with said target molecules through

30   the preselected structure;
          (c)   separating the morphatides performing the preselected
                  or desired function or binding or associating through
                  the preselected structure, from the library of
                  morphatides and target molecules; thereby identifying

35   one or more complexes from a library of complexes,
                  wherein said complex or complexes are selected for

their ability to perform a preselected or desired function on a target molecule or by having a pre-selected structure.

5   3.   The method of either of claims 1 or 2, wherein the separation of step (c) is performed by either (a) separating the morphatides which do not perform the preselected or desired function or which do not bind or associate through a pre-selected structure or (b)
10   separating the morphatides which perform the preselected or desired function or which bind or associate through a pre-selected structure.

  4.   The method of either of claims 1 or 2, wherein said target
15   molecule is bound to a solid support.

  5.   The method of either of claims 1 or 2, wherein the preselected or desired function performed by the complex(es) on a target molecule is selected from the group
20   consisting of binding to or associating with said target molecule; reacting with said target molecule and changing the property of said target molecule; having an affinity for and binding to a specific ligand; performance of a biological activity, wherein said biological activity is
25   selected from the group consisting of antimicrobial activity, antitumor activity, enzyme inhibiting activity, enzyme enhancing activity, receptor binding activity, growth promotion activity, antibody binding activity; formation of a biofilm; enzymatic activity, immune
30   modulating activity, cell signaling activity, polymerizing activity, and encapsulating activity.

  6.   The method of either of claims 1 or 2, wherein said contacting, binding or association is selected from the
35   group consisting of multiple complexes acting on a single target molecule, a single complex acting on multiple target

76

molecules, components of one or more complexes acting on multiple target molecules, components of one or more complexes acting on a single target molecule, and multiple complexes acting on multiple target molecules.

5

7.   The method of either of claims 1 or 2, wherein said scaffolding component comprises naturally occurring nucleotides, novel or unique bases, base analogs; synthetically generated nucleic acids, nucleic acid like molecules, and nucleic acid analogs; or any combination thereof.

10

8.   The method of claim 7, wherein the nucleic acid like molecule is a difluorotoluene or related deoxynucleoside.

15

9.   The method of either of claims 1 or 2, wherein said scaffolding component consists of subunits which are capable of being incorporated by one or more nucleic acid polymerases or reverse transcriptases and which can, when polymerized, generate hybridizable polymers with hydrogen bonding or hybridizable polymers without hydrogen bonding.

20

10.   The method of either of claims 1 or 2, wherein said scaffolding component comprises nucleic acids having regions of conserved sequences and one or more regions of randomized sequences.

25

11.   The method of either of claims 1 or 2, wherein the scaffolding component(s) are comprised of two fixed regions of nucleotides and one region of randomized nucleotides between the two fixed regions.

30

12.   The method of claim 1, wherein the linker component is associated to a base of the scaffolding component either before or after the scaffolding component is made.

35

13. The method of either of claims 10 or 11, wherein the randomized region is comprised of:
(a) three of the four bases occurring with similar frequency; and

5

(b) one of the four bases occurring at a rare frequency.

14. The method of claim 13, wherein one of the bases occurring with similar frequency is associated with or binds with the linker component.

10

15. The method of claim 13, wherein one of the four bases occurring at a rare frequency is associated with or binds with the linker component.

15 16. The method of claim 14, wherein the position of the base with the linker attached is determined by nucleotide sequencing or mass spectrophotometry.

17. The method of claim 7, wherein each scaffolding component

20   comprises more than one different nucleic acid base being attached to a linker component, said nucleic acid base being incorporated into the scaffolding component either during PCR amplification or during synthesis of the nucleic acids.

25

18. The method of claim 17, wherein the incorporated nucleic acid base to which the linker component is attached is a rare base.

30 19. The method of claim 17, wherein the base to which the linker is attached is modified, said modification being by chemical reaction either before or after incorporation during PCR amplification or during synthesis of the nucleic acids.

35

20.   The method of claim 17, wherein the incorporated nucleic
       acid base to which the linker component is attached is a
       different modified base, said base being any of the four
       bases or analogs of said bases, said base being modified by
5   a reaction.

21.   The method of claim 17, wherein the incorporated base to
       which the linker component is attached is located
       internally in the scaffolding component.

10

22.   The method of claim 1, wherein one or more of said linker
       components are either reversible or non-reversible, and
       wherein one or more of said linker components comprise
       reversibly connectable components or parts.

15

23.   The method of claim 1, wherein one or more of said linker
       components cannot be amplified in vitro or in vivo.

24.   The method of either of claims 1 or 2, wherein one or more
20   of said scaffolding components associated with one or more
       of said linker components is amplifiable in vitro or in
       vivo.

25.   The method of claim 1, wherein said one or more of said
25   linker components connected to one or more of said agent
       molecules cannot be amplified in vitro or in vivo.

26.   The method of claim 1 or 2, wherein the entire morphatide
       is amplifiable.

30

27.   The method of claim 30, wherein a first linker component
       either reversibly or non-reversibly associated with a
       scaffolding component and a second linker component either
       reversibly or non-reversibly associated with an agent
35   molecule are connected together to generate a scaffolding

79

component linked to an agent molecule by the connectable
first and second components of said linker component.

28.   The method of claim 1, wherein the linker component is
       selected from the group consisting of a phenyl-boronic acid
       linker, a thio linker, and a biotin-streptavidin linker.

29.   The method of claim 28, wherein the thio linker is cysteine.

30.   The method of claim 2, wherein the scaffolding component is
       associated to one or more agent molecules, wherein said
       agent molecule is a peptide by a peptide bond.

31.   The method of claim 1, wherein the linker component is
       selected from the group consisting of a nucleic acid
       binding protein and a chelating molecule.

32.   The method of claim 1, wherein the linker component is
       either bound covalently to either the scaffolding component
       or to the agent molecule or the linker component is bound
       noncovalently to either the scaffolding component or to the
       agent molecule.

33.   The method of either of claims 1 or 2, wherein said agent
       molecules are selected from the group consisting of
       naturally occurring polymers, synthetically generated
       polymers, and non-polymeric molecules.

34.   The method of either of claims 1 or 2, wherein the library
       of complexes is prepared by:

       (a) coupling the linker molecules or components of the
       linker molecules to either the scaffolding components, to
       form scaffolding component-linker molecules or to the agent

molecules, to form agent molecule-linker molecules; and
(b) generating a linkage between the scaffolding component-
linker molecules and the agent molecules or between the
scaffolding components and the agent molecule-linker
molecules to yield the complexes, thereby preparing a
library of complexes.

35. The method of claim 34, wherein said scaffolding components
are prepared for coupling to linker molecules via chemical
reaction yielding modified nucleotides.

36. The method of claim 21, wherein said chemical reaction
involves treating the scaffolding components with one or
more mutagens to add one or more base specific or non-
specific adduct(s), resulting either in adducted
scaffolding molecules, that enable increased reactivity of
the base to the linker or directly to the agent molecules
or in adducted scaffolding molecules, said adduct acting as
either a linker or an agent molecule.

37. The method of either of claim 36, wherein the adducted
scaffolding components are amplifiable.

38. The method of either of claim 36, wherein said mutagen is
UV light, any other nucleic acid mutagen, or a nucleic acid
binding protein.

39. The method of claim 21, wherein said chemical reaction
involves treating scaffolding components with Maxam &
Gilbert based chemistries to generate increased reactivity
of one or more bases to a linker or to an agent molecules.

40. The method of claim 34, wherein said scaffolding components
are prepared for coupling to the linker molecules via a
non-chemical reaction yielding modified nucleotides.

81

41.   The method of claim 40, wherein said non-chemical reaction
      is an enzymatic reaction.

42.   The method of either of claims 1 or 2, said method further
      comprising after step (b):
      (a) disassociating the scaffolding component of the complex
      performing the preselected or desired function from the
      agent molecule or molecules;
      (b) generating modified scaffolding components;
      (c) associating the different scaffolding molecules with
      agent molecules to generate different morphatides;
      (d) rescreening the different morphatides by repeating
      steps (b) and (c) of claims 1 or 2 to identify new desired
      candidate morphatides.

43.   The method of claim 42, wherein said modification of
      scaffolding components occurs via a random or directed
      mutagenesis technique.

44.   The method of claim 43, wherein said random or directed
      mutagenesis techniques are selected from the group
      consisting of error-prone PCR or sexual PCR by performing
      a suitable number of cycles on the scaffolding components,
      resulting in one or more base changes in some percentage of
      the scaffolding components; cassette mutagenesis; and site
      directed mutagenesis.

45.   The method of claim 42, wherein one or more of said agent
      molecules in step (c) are different from the agent
      molecules utilized in the morphatides of the prior round of
      screening for identification of morphatides performing the
      preselected or desired function.

46.   The method of either of claims 1 or 2, for identifying a
      different morphatide further comprising:
      (a) separating the scaffolding components from the agent

molecules;

(b) performing a suitable number of cycles of error prone PCR on the scaffolding components, resulting in one or more base changes in some percentage of the scaffolding component;

(c) reconnecting the scaffold component to the agent component; and

(d) repeating steps (a) through (d) of claims 1 or 2, thereby identifying a different morphatide.

47. The method of claim 42, wherein the morphatide comprises a linker component, wherein in step (a) one part of a linker remains attached to the scaffold component and another part of the linker remains attached to the agent molecule and wherein in step (c) both parts of the linker are connected, thereby reconnecting the scaffold component to the agent component or wherein the connection between the agent molecule and the scaffolding component is by a plurality of the linker components.

48. The method of claim 42, wherein the scaffolding components are characterized by cloning and nucleotide sequencing .before reattachment of the agent molecule in step (c).

49. The method of either of claims 1 or 2, further comprising:
(a) creating a mimic of the identified morphatide; and
(b) using the mimic for a desired application.

50. The method of either of claims 1 or 2, further comprising:
(a) separating scaffolding components with attached linker components or parts of the linker components from the agent molecules of the previously identified Morphatides;
(b) combining the scaffolding components with attached linker components or parts thereof with scaffolding components comprising a same nucleic acid sequence as the scaffolding components, said nucleic acid sequence not

being attached to or associated with a linker components or parts thereof, thereby resulting in nucleic acid sequences without the one or more linker sites;

(c) using sexual PCR to fragment and reassemble the nucleic acid sequences, resulting in elimination of linker component sites which do not contribute to the binding of the morphatide, thereby generating new scaffolding components similar but not identical to the scaffolding components of step (a);

(d) reattaching or association agent molecules to   the new scaffolding components of step (c), thereby generating another set of Morphatides.

51. A method of identifying a presence of a substance in a sample from a subject, comprising:

(a) obtaining a sample;

(b) contacting the sample with one or more types of morphatide identified by the method of either of claims 1 or 2 so as to form a complex between the morphatide and the substance present in the sample;

(c) detecting the complex formed in step (b),

thereby identifying the presence of the substance in the sample from the subject.

52. The method of claim 51, wherein step (c) is performed by PCR amplification, ethidium bromide staining or labeling selected from the group consisting of radioactive isotope, enzyme, dye, biotin, a fluorescent label, a chemiluminescent label and a ligand.

53. The method of claim 51, wherein the detection of the complex formed in step (c) comprises identification of an occurrence selected from the group consisting of binding to or associating with a target molecule; having an affinity for and binding to a specific ligand; performance of a

biological activity, wherein said biological activity is selected from the group consisting of antimicrobial activity, antitumor activity, enzyme inhibiting activity, enzyme enhancing activity, receptor binding activity, growth promotion activity, antibody binding activity; formation of a biofilm; enzymatic activity, immune modulating activity, cell signaling activity, polymerizing activity, and encapsulating activity.

54. A method of diagnosing a subject wherein detection of a complex in step (c) of claim 51 is indicative of a disease.

55. The method of claim 51, wherein the sample is a body fluid or a tissue specimen.

56. The method of claim 55, wherein the body fluid is selected from the group consisting of blood, serum, plasma, urine, saliva, nasal mucosal discharge, vaginal mucosal discharge, anal mucosal discharge, peritoneal fluid, cerebro-spinal fluid, and lymphatic fluid.

57. The method of claim 51, wherein the substance whose presence is identified is selected from the group consisting of hormones, enzymes, proteins, cancer/tumor cells, pathogens, and drugs.

58. The method of claim 51, wherein the subject is bacterial cells, a plant, a microbe, an insect, a fish, or a mammal.

59. The method of claim 58, wherein the mammal is a human.

60. A morphatide capable of effectively binding to, crosslinking with, or reacting with multiple types of molecules.

61. A morphatide capable of effectively binding to, crosslinking

with, or reacting with one type of molecule.

62. The morphatide of either of claims 60 or 61, wherein binding to, crosslinking with, or reacting with the molecules is selected from the group consisting of binding to or associating with a target molecule; having an affinity for and binding to a specific ligand; performance of a biological activity, wherein said biological activity is selected from the group consisting of antimicrobial activity, antitumor activity, enzyme inhibiting activity, enzyme enhancing activity, receptor binding activity, growth promotion activity, antibody binding activity; formation of a biofilm; enzymatic activity, immune modulating activity, cell signaling activity, polymerizing activity, and encapsulating activity.

63. A composition comprising a morphatide effective to treat a subject and a pharmaceutically acceptable carrier.

64. The method of administering the composition of claim 63, wherein the administration is intravenous, intraperitoneal, intrathecal, intralymphatical, intramuscular, intralesional, parenteral, epidural, subcutaneous; by infusion, liposome-mediated delivery, aerosol delivery; topical, oral, nasal, anal, ocular or otic delivery.

65. The morphatide of either of claims 60 or 61, conjugated to a therapeutic agent.

66. The morphatide of claim 65, wherein the therapeutic agent is a radioisotope, toxin, toxoid, or chemotherapeutic agent.

67. A composition comprising the conjugated morphatide of claim 65 and a pharmaceutically acceptable carrier, wherein the morphatide is selected from either a morphatide which is

capable of being degraded or a morphatide which is incapable of being degraded after administration to a subject.

5   68.   A morphatide labeled with a detectable marker.

     69.   The morphatide of claim 68, wherein the detectable marker is selected from the group consisting of a radioactive isotope, enzyme, dye, biotin, a fluorescent label, a
10   chemiluminescent label and a ligand.

     70.   The composition of claim 67, wherein the morphatide is incapable of being degraded further comprising a stabilizer molecule for increasing the half-life of the morphatide in
15   the blood stream.

     71.   The composition of claim 70, wherein the stabilizer is polyethyleneglycol.

20   72.   A method of treating a subject with the composition of either of claims 63 and 67.

     73.   A method of drug delivery to a target in the body of a subject comprising administration to a subject of the
25   composition of either of claims 63 and 67, thereby delivering the drug to the target.

     74.   The method of claim 67, wherein the degradation is performed by either of a nuclease or protease.
30
     75.   A method of drug delivery to a target in the body of a subject comprising administration to a subject of the composition of claim 63, wherein the morphatide is incapable of being degraded or is slowly degraded after
35   administration to the subject, thereby delivering the morphatide-bound drug to the target.

76. The method of claim 75, wherein the morphatide-bound drug
    is administered, wherein the administration is intravenous,
    intraperitoneal, intrathecal, intralymphatical,
5   intramuscular, intralesional, parenteral, epidural,
    subcutaneous; by infusion, liposome-mediated delivery,
    aerosol delivery; topical, oral, nasal, anal, ocular or
    otic delivery.

10 77. The method of claim 1, wherein the morphatide is capable of
    binding to any component of an antibody molecule, said
    antibody having a constant and variable region.

78. A morphatide identified according to the method of claim 1.
15
79. A morphatide identified according to the method of claim 42.

80. A morphatide identified according to the method of claim 46.

20 81. A morphatide identified according to the method of claim 50.

82. A method of increasing the binding affinity of a morphatide
    for a selected target molecule, said morphatide comprising
    a nucleic acid scaffold and one or more agent molecules;
25 said   method   comprising:
    (a)   amplifying   the   nucleic   acid   scaffold   of   the
    morphatide;
    (b)   generating   modified   scaffolding   components;
    (c)   associating the different scaffolding components with
30 said   agent   molecules   to   generate   different   morphatides;
    (d)   screening the morphatides for ability to bind to the
    selected   target;   and
    (e)   separating morphatides with an increased binding
    affinity for said target.

88

83. A method of increasing the binding affinity of a morphatide
    for a selected target molecule, said morphatide comprising
    a nucleic acid scaffold and one or more agent molecules;

5   said   method   comprising:
    (a) performing a suitable number of cycles of error prone
    PCR on the scaffolding component of the morphatide,
    resulting in one or more base changes in some percentage of
    the   scaffolding   component;

10 (b) associating the different scaffolding components with
    said agent molecules to generate different morphatides;
    (c) screening the morphatides for ability to bind to the
    selected   target;   and
    (d) separating morphatides with an increased binding

15 affinity for said target.

84. A method of increasing the binding affinity of a morphatide
    for a selected target molecule, said morphatide comprising
    a nucleic acid scaffold, a linker component and one or more

20 agent   molecules;   said   method   comprising:
    (a) amplifying the scaffold component of the morphatide;
    (b) associating said scaffold components with linker
    components   or   parts   thereof;
    (c) combining the scaffolding components with attached

25 linker components or parts thereof with scaffolding
    components comprising the same nucleic acid sequence as the
    scaffolding components, said nucleic acid sequence not
    being attached to or associated with a linker components or
    parts thereof, thereby resulting in nucleic acid sequences

30 without   the   linker   sites;
    (d) using sexual PCR to fragment and reassemble the nucleic
    acid sequences, resulting in elimination of linker
    component sites which do not contribute to the binding of
    the morphatide, thereby generating new scaffolding

35 components similar but not identical to the scaffolding

89

components   of   step   (a);

(e) associating agent molecules to the new scaffolding components of step (d), thereby generating a different set of   morphatides;

5   (f) screening the morphatides for ability to bind to the selected   target;   and

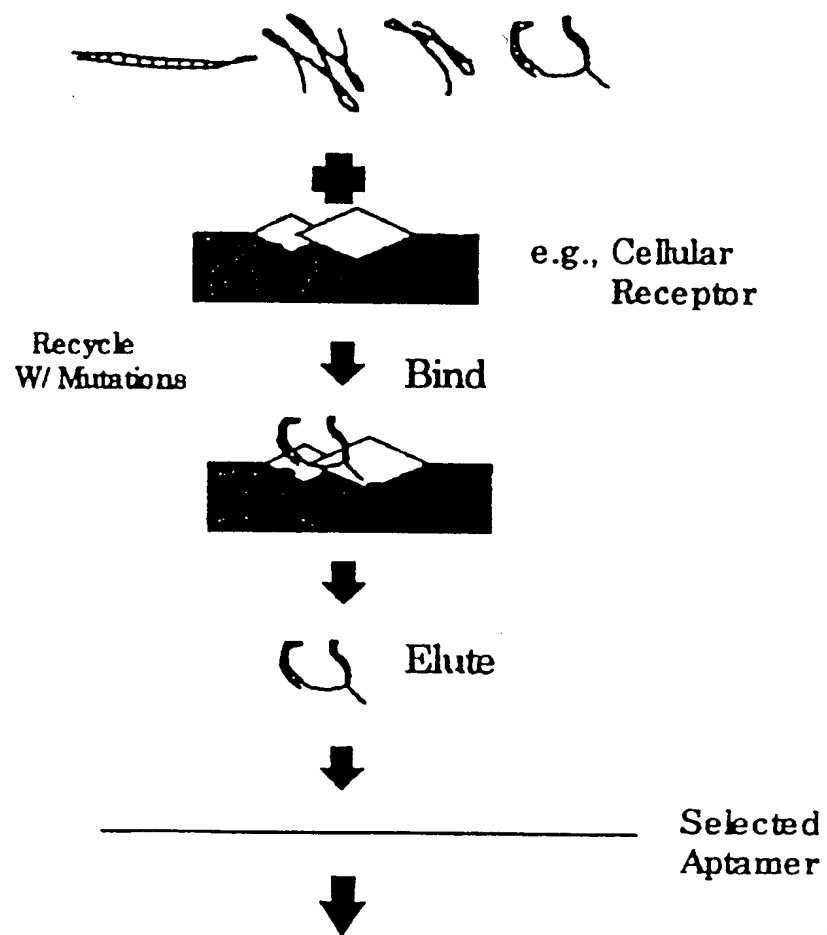(g) separating morphatides with an increased binding affinity for said target.

90

FIG. 1A

FIG. 1A
FIG. 1B

Aptamer Selection

e.g., Cellular
Receptor

Recycle
W/ Mutations   ⬇ Bind

⬇

⬇ Elute

⬇

_____ Selected
Aptamer

⬇

FIG. 1B

Aptamer Generation

Aptamers

FIG. 2A

FIG. 2A
FIG. 2B

Morphotide based combinatorial Chemistry: (shown only in 2 dimensions)

Template

Sloppy amplification w/ coupling nucleotides

Create morphotide by binding

Select for binding morphotide
(bind and wash)

Release chemical group

(Continued on next)

FIG. 2B

Sloppy amplification w/ coupling nucleotides

Create morphotide by binding

Select for binding morphotide (bind and wash)

Release chemical group

(Continued next round of amplification and selection)

5/7

FIG. 3A

| FIG. 3A | FIG. 3B |

Reversible Coupler

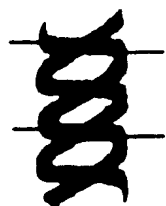# Shape & Structure Library
(Adaptamer)
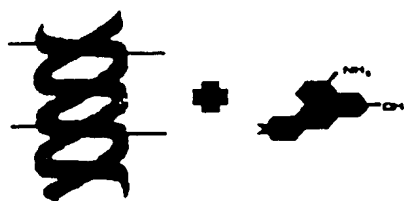
Coupling Step
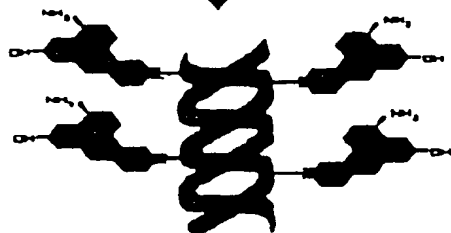
Bind
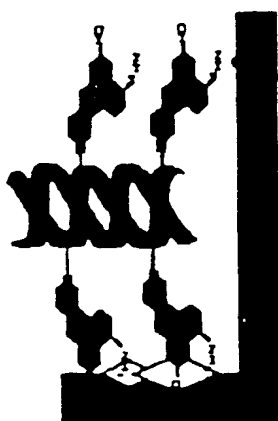
Elute w/
pH Shift

PCR
Mutate

e.g., Cellular
Receptor

FIG. 3B

FIG. 4

Candidate bioconjugate:
Phenylboronic Acid

$pK_A = 4.2$
$K_D = 10^{15}$

REVERSIBLE COMPLEX 1.5 UNITS OF PH ≡ 0.5 OXIDATION BY HYDROLYSIS

CAN TRAP AT PH 8 & CAN RELEASE